DISSERTATIONES SCHOLA DOCTORALIS SCIENTIAE CIRCUMIECTALIS, ALIMENTARIAE, BIOLOGICAE. UNIVERSITATIS HELSINKIENSIS

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SPRING BLOOM DYNAMICS IN THE BALTIC SEA: FROM THE ENVIRONMENT TO MACROELEMENTS AND MICROBIAL INTERACTIONS

FACULTY OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES
DOCTORAL PROGRAMME IN INTERDISCIPLINARY ENVIRONMENTAL SCIENCES
UNIVERSITY OF HELSINKI

Faculty of Biological and Environmental Sciences
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Spring bloom dynamics in the Baltic Sea: From the environment to macroelements and microbial interactions

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Division of Ecosystem and Environment Research Program
Aquatic Sciences

Academic dissertation

Doctoral thesis, to be presented for public examination with the permission of the Faculty of Biological and Environmental Sciences of the University of Helsinki, in Auditorium Metsätalo (forest house) room 6, Unioninkatu 40, on the 20th of August, 2020 at 10 o'clock.

Helsinki 2020

Research locations:	Finnish Environment Institute (Marine Research Centre) & Tvärminne Zoological Station (University of Helsinki)
Doctoral program:	Doctoral program in interdisciplinary environmental Sciences (DENVI)
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Custos:	Kimmo Kahilainen – University of Helsinki

Cover design:	Omar Escalante (arcticframe.com)
Publication series:	Dissertationes Schola Doctoralis Scientiae Circumiectalis, Alimentariae, Biologicae. Universitatis Helsinkiensis
Printing house:	Hansaprint Oy
Printing location:	Vantaa
Printing year:	2020
ISBN / ISSN (paperback):	978 – 951 – 51 – 6285 – 4 / 2342-5423
ISBN / ISSN (PDF):	978 – 951 – 51 – 6286 – 1 / 2342-5431
Ethesis:	https://ethesis.helsinki.fi/en/

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“I was just guessing at numbers and figures
Pulling your puzzles apart
Questions of science, science and progress
Do not speak as loud as my heart”

The Scientist, Coldplay (2002)

Abstract

The Baltic Sea is a substantial brackish water system, and due to its shallow average depth, physicochemical dynamics affecting, for instance, phytoplankton growth, are controlled by atmospheric forces. This unique ecosystem is threatened by environmental changes, with implications for the water quality. Eutrophication remains the major challenge for the Baltic Sea ecosystem. The increases in nitrogen (N) and phosphorus (P) are not proportional to the carbon (C) input, which might cause imbalances in seston C:N:P stoichiometry and affect biogeochemical cycles. In turn, plankton growth, food web dynamics, and nutrient- / C-cycling, which plays a pivotal role in climate change, will be affected. The ongoing eutrophication supports the formation of high phytoplankton biomass, enhancing the spreading of anoxic bottom waters, affecting benthic communities, which might be worsened by an extended annual growing season caused by increasing sea surface temperatures (SST). Higher SST and effects of eutrophication are known to support harmful algal blooms such as the occurrence of toxic dinoflagellates and cyanobacteria in the Baltic Sea during summer.

The spring bloom is the most productive period of the year (~50 % annual C-fixation), driven by high inorganic nutrient concentrations and improving light conditions. The co-existence of cold-water diatoms and dinoflagellates during this period is characteristic of the Baltic Sea. Heterotrophic bacteria will become more abundant due to increased concentrations of allochthonous organic matter, which could reduce the phototrophic contribution already in spring. The health of arctic and sub-arctic ecosystems is highly dependent on environmental factors such as temperature affecting, for example, sea ice conditions. Climatically driven changes have already caused variations in the plankton community structure during the Baltic Sea spring bloom. These alterations have led to lower diatom-dinoflagellate-proportions (DDP), affecting food web dynamics and biogeochemical cycles. Therefore, shifts in the community composition may have far-reaching ecosystem consequences. The dominant phytoplankton group defines the quantity and quality of bioavailable (labile) dissolved organic carbon (DOC), providing different niches for heterotrophic bacteria. Furthermore, environmental factors affect the abundances and community compositions of both phototrophic and heterotrophic plankton. Microzooplankton is particularly crucial in linking the microbial loop with higher trophic levels, and in the recycling of inorganic nutrients. For example, heterotrophic ciliates become more abundant at elevated temperatures and can significantly reduce the biomass of bacteria and phytoplankton. The Baltic Sea spring bloom is dominated by phytoplankton, while the grazer community is not well developed yet. Thus, considering the ongoing climatic changes, it can be expected that the share of microzooplankton and, in turn, the partially unaccounted biomass will be higher at elevated temperatures (e.g., in summer). Temporal and spatial shifts of zooplankton populations, affecting higher trophic levels, could be the consequence.

<u>The main objectives of this study:</u>

- The identification of environmental drivers shaping the nano- and microplankton community composition.
- To study the effects of changing communities and bloom phases on seston nutrient stoichiometry.
- The investigation of bacterioplankton responses to changing dominance patterns of diatoms and dinoflagellates.
- To reveal the proportion of microzooplankton during the spring bloom.

For this, data from research cruises (chapters 1 and 3) in the Baltic Sea and experiments carried out at Tvärminne Zoological Station (TVZ, University of Helsinki) on the south-west coast of Finland (chapter 2) were used. Surface water samples (z = 3 m) for the analysis of nutrients (inorganic and organic), biomass, community composition, and primary production amongst others were collected from seven sub-basins and different spring bloom phases (April / May) during four cruises on-board R/V Aranda (2013 to 2016). The Gulf of Finland (GOF) and the Baltic Proper were the main study areas. The two experiments (2012 and 2013) at TVZ (also GOF) took place in February / March. The water was collected from 20 m under the sea ice and featured low biomass and high inorganic nutrient concentrations (winter conditions). In addition to nutrients and phototrophic variables, samples were taken to determine bacterial abundances, production, and community composition (BCC). The main difference between these two approaches was the fact that the cruise data comprise natural mixed communities, and samples from different bloom phases represent an extract of the spring bloom. In contrast, dominance patterns were artificially shifted by adding cultured

strains to the natural pre-bloom community for the experiments. Furthermore, the bacterial growth phase was induced by increasing the temperature, and the course of the bloom was followed in 20 L mesocosms. The determination of nutrient and chlorophyll *a* (Chl *a*) concentrations as well as the microscopy counting strategy (field data) were based on in-house protocols of the Finnish Environment Institute (SYKE), certified by the Finnish Accreditation Service (FINAS). Some of the main methods were explicitly chosen for the study area (Baltic Sea), the sampling season (spring bloom), and the database (Hertta, SYKE) that was used (plankton community composition, upper mixed layer depth, definition of bloom phases, bacterial production, and gross primary production). Various multivariate and other statistical analyses were applied to study different aspects of the presented studies. In addition to the different chapters, three cold-water dinoflagellates, usually not identified to species-level, were distinguished, and their biomass-contributions investigated (cruise data, unpublished). The corresponding species (*Gymnodinium corollarium*, *Biecheleria baltica*, and *Apocalathium malmogiense*) can co-occur during the Baltic Sea spring bloom but are not separable by traditional microscopy of Lugol-preserved samples due to their similar appearance. Thus, this group is referred to as DinoComplex throughout this summary. Two different methods for the identification and quantification of the three species were applied: inverted epifluorescence microscopy of stained (fluorescence brightener) cells and quantitative polymerase chain reaction (qPCR), using species-specific primers and fluorescent (Taqman®) probes.

Environmental factors, mostly temperature and the concentration of inorganic nutrients, defined the nano- and microplankton community composition (phototrophs and heterotrophs) and affected the collective physiology as well as the biomass. Combined, *Mesodinium rubrum*, *Peridiniella catenata*, the DinoComplex, and heterotrophic ciliates dominated the biomass in the cruise data (n = 119). The experiments have shown that lower DDP´s reduce the bacterial activity (protein- and DNA-synthesis) and alter the BCC due to decreasing availability of diatom-derived dissolved organic matter (DIADOM). Additionally, a predominant diatom-species has shown specific effects on bacterial responses, i.e., *Chaetoceros wighamii* dominance yielded the highest bacterial activity. In the field data, the community composition explained 19 % (average) of shifts in seston C:N:P ratios, which were significantly different between some of the bloom phases.

The presented findings highlight the importance of species-level identification (e.g., DinoComplex) and the introduction of a microzooplankton monitoring program. The fact that the most relevant diatom (*Thalassiosira baltica*) contributed <10 % to the biomass (relative contribution, n = 119), combined with high dinoflagellate-proportions throughout the bloom, agrees with previous findings on the increase of dinoflagellates over diatoms in several sub-basins. Not only variations in the community composition, but also different growth phases (physiology) of the bloom affected the interactions between phyto- and bacterioplankton. For instance, high proportions of *T. baltica* (equivalent to ~6 µg Chl *a* L^{-1}) were associated with low primary production (field data, presented study) as well as low bacterial activity (field data, not shown). At the bloom peak, the cells are less active, but not decaying and excreting larger amounts of labile DOM, yet. Thus, a decreasing availability of DIADOM will cause a decrease in the energy efficiency of the microbial food web.

Comparing the C:N:P ratios from the field study and the experiments revealed that the absolute dominance of either diatoms or dinoflagellates lead to more clear connections to the intracellular element composition. However, there was no significant relationship between the DDP and seston C:N:P ratios, and thus, it was concluded that the overall community activity supersedes the effect of different dominance-patterns on seston stoichiometry in mixed communities. Species-specific effects on bacterial responses and seston stoichiometry, with implications for biogeochemical cycles, are challenging to study in diverse communities. Seston ratios that were clearly affected by the community composition, namely Chl *a*:C (and its fixed range), C:Si, and N:Si, could be predicted with relatively high certainty for the spring bloom. The Chl *a*:C ratio can be used as a proxy for phototrophic and heterotrophic production and to study C-budgets. For example, C:Si indicates the diatom-proportion of the seston, which could be interesting for modeling studies. Due to a high inter-annual variability in seston ratios, season-specific findings should be combined to estimate future developments of, for example, the annual primary production and C-fluxes.

Tiivistelmä (translated by Mari Vanharanta in collaboration with Samu Elovaara)

Itämeri on laaja murtovesialue, jonka ainutlaatuista ekosysteemiä uhkaavat veden laatuun vaikuttavat ympäristömuutokset. Rehevöityminen on Itämeren suurin ongelma. Se voi aiheuttaa epätasapainoa sestonin (vesipatsaan elollinen ja eloton orgaaninen aines) C:N:P-stoikiometriassa ja vaikuttaa biogeokemiallisiin kiertoihin. Vaikutukset yltävät ilmastonmuutoksen kannalta keskeisiin mekanismeihin kuten planktonin kasvuun, ravintoverkkojen dynamiikkaan sekä ravinteiden ja hiilen kiertoon. Rehevöityminen lisää kasviplanktonbiomassan muodostumista, minkä seurauksena hapettomien pohjasedimenttien pinta-ala kasvaa. Samalla pohjaeläinyhteisöjen elinmahdollisuudet heikkenevät, ja rehevöitymisen vaikutukset saattavat yhä pahentua tulevaisuudessa. Ilmaston lämpenemisen ja rehevöitymisen vaikutusten tiedetään lisäävän haitallisten leväkukintojen, kuten myrkyllisten panssarisiimalevien ja syanobakteerien, esiintymistä Itämerellä kesäisin. Kevätkukinta on vuoden tuottavin jakso, jota epäorgaanisten ravinteiden pitoisuus ja valaistusolosuhteet säätelevät. Kylmän veden piilevien ja panssarisiimalevien rinnakkaiselo kevätkukinnan aikaan on ominaista Itämerelle. Toisenvaraisten bakteerien määrä kasvaa valuman mukana tulevan orgaanisen aineksen lisääntyessä, mikä voi vähentää yhteyttävien levien osuutta jo keväällä. Arktisten ja subarktisten ekosysteemien hyvinvointi on riippuvainen ympäristötekijöistä, kuten lämpötilasta, joka vaikuttaa esimerkiksi merijään muodostumiseen. Ilmastomuutos on jo aiheuttanut muutoksia Itämeren keväisen planktonyhteisön lajikoostumuksessa. Nämä muutokset ovat johtaneet matalampiin pii- ja panssarisiimalevien välisiin suhteellisiin osuuksiin, vaikuttaen ravintoverkkoon ja biogeokemiallisiin kiertoihin. Siksi yhteisökoostumuksen muutoksilla voi olla ekosysteemitason seurauksia. Hallitseva kasviplanktonryhmä määrittelee biologisesti käyttökelpoisen liuenneen orgaanisen hiilen määrän ja laadun tarjoten erilaisia elinmahdollisuuksia toisenvaraisille bakteereille.

Ympäristötekijät vaikuttavat sekä oma- että toisenvaraisen planktonin määrään ja yhteisöjen lajikoostumukseen. Mikroeläinplankton on erityisen tärkeä linkki ravintoverkossa mikrobien ja korkeampien ravintoketjun tasojen välillä sekä epäorgaanisten ravinteiden kierrossa. Kasviplankton hallitsee kevätkukinnan aikaista eliöyhteisöä, kun laiduntajayhteisö ei ole vielä ehtinyt kehittyä. Ripsieläimet voivat kuitenkin olla tärkeitä bakteerien ja kasviplanktonin laiduntajia erityisesti lämpötilan kohottua. Siksi vielä toistaiseksi piilossa olevan mikroeläinplanktonin osuus kokonaisyhteisöstä oletettavasti kasvaa elinympäristön lämmetessä, mikä aiheuttaa ajallisia ja alueellisia muutoksia eläinplanktonpopulaatioissa ja vaikuttaa korkeampiin ravintoketjun tasoihin.

Tutkimuksen päätavoitteet:

- Tunnistaa planktonyhteisön (<100 µm) koostumukseen vaikuttavat ympäristötekijät.
- Tutkia muuttuvien eliöyhteisöjen ja kukintavaiheiden vaikutuksia sestonin ravinnestoikiometriaan.
- Tutkia bakteeriplanktonin vastetta muuttuvaan piilevien ja panssarisiimalevien suhteeseen.
- Selvittää mikroeläinplanktonin osuutta kevätkukinnassa.

Lisäksi, erotettiin toisistaan kolme kylmän veden panssarisiimalevälajia (Gymnodinium corollarium, Biecheleria baltica ja Apocalathium malmogiense), joita ei tavanomaisesti määritetä lajitasolle, ja näiden kolmen lajin biomassaosuudet selvitettiin.

Ympäristötekijät, erityisesti lämpötila ja epäorgaanisten ravinteiden pitoisuus, määrittivät planktonyhteisön (<100 µm) koostumusta sekä vaikuttivat sen fysiologiaan ja kokonaisbiomassaan. Ripsieläinten ja panssarisiimalevien yhteisbiomassa hallitsi kenttäaineiston kokonaisbiomassaa. Kokeet ovat osoittaneet, että matala piilevien ja panssarisiimalevien välinen suhteellinen osuus vaikuttaa bakteerien aktiivisuuteen ja yhteisön koostumukseen alhaisemman piilevistä peräisin olevan liuenneen orgaanisen aineksen määrän vuoksi. Lisäksi, vallitseva piilevä, Chaetoceros wighamii, ylläpiti korkeinta bakteeriaktiivisuutta. Kenttäaineiston perusteella yhteisön koostumus selitti keskimäärin 19% sestonin C:N:P-suhteen muutoksista. Nämä suhteet vaihtelivat kukintavaiheen mukaan.

Tutkimushavainnot korostavat lajitason tunnistustarkkuuden tärkeyttä ja suosittavat mikroeläinplanktonin lisäämistä seurantaohjelmaan. Kaikkein merkittävimmän piilevän (Thalassiosira baltica) suhteellinen osuus oli vain alle 10 % kokonaisbiomassasta, minkä lisäksi panssarisiimalevien määrä oli kenttäaineiston perusteella korkea koko kukinnan ajan. Nämä tiedot tukevat aikaisempia havaintoja siitä, että panssarisiimalevien määrä suhteessa piileviin on kasvanut

useilla Itämeren altailla. Yhteisön koostumuksen lisäksi myös kukintavaihe vaikuttaa kasviplanktonin ja bakteeriyhteisön väliseen vuorovaikutukseen. Kukintahuipun aikaan solut ovat verraten epäaktiivisia ja käyttökelpoisen liuenneen orgaanisen aineksen määrä on melko alhainen. Piilevistä peräisin olevan liuenneen orgaanisen aineksen väheneminen heikentää mikrobiyhteisön energiatehokkuutta. Kenttätutkimuksissa tai kokeissa ei kuitenkaan havaittu merkitsevää yhteyttä piilevien tuottaman liuenneen orgaanisen aineen ja sestonin C:N:P-osuuksien välillä. Siten voidaan todeta, että kokonaisyhteisön aktiivisuus peitti alleen lajikoostumuksen vaikutukset sestonin stoikiometriaan. Sestonin C:N:P-osuudet pystyttiin ennustamaan yhteisörakenteen perusteella suhteellisen suurella varmuudella kevätkukinnan aikaan. Klorofylli a:n ja hiilen välistä suhdetta voidaan käyttää omavaraisen ja toisenvaraisen tuotannon indikaattorina ja hiilibudjettien tutkimisessa. Hiilen ja piin suhde puolestaan kuvaa piilevien osuutta sestonista, mikä voi hyödyttää mallinnustutkimusta. Sestonin sisältämien ravinneosuuksien korkean vuosittaisen vaihtelun takia kausikohtaiset havainnot pitäisi yhdistää pitkän aikavälin tutkimuksiin.

List of original publications and author contributions

The presented thesis is composed of three chapters, referred to as chapters 1, 2, and 3 in the following (***** = corresponding authors).

Chapter 1 (article in press)

Lipsewers T *, Klais R, Camarena-Gómez MT, Spilling K (2020): Effects of different plankton communities and spring bloom phases on seston C:N:P:Si:Chl *a* ratios in the Baltic Sea. *Marine Ecology Progress Series*, https://doi.org/10.3354/meps13361

Chapter 2 (published article)

Camarena-Gómez MT *****, **Lipsewers T**, Piiparinen J, Eronen-Rasimus E, Perez-Quemaliños D, Hoikkala L, Sobrino C, Spilling K (2018): Shifts in phytoplankton community structure modify bacterial production, abundance, and community composition. *Aquatic Microbial Ecology* 81:149-170.

Chapter 3 (published article)

Lipsewers T, Spilling K ***** (2018) Microzooplankton, the missing link in Finnish plankton monitoring programs. *Boreal Environmental Research* 23:127-137.

Author contributions

Author initials: **TL = Tobias Lipsewers**, KS = Kristian Spilling, RK = Riina Klais, TC = Maria Teresa Camarena-Gómez, HK = Harri Kuosa, JP = Jonna Piiparinen, DP = Daniel Perez-Quemaliños, CS = Cristina Sobrino, EER = Eeva Eronen-Rasimus

	Chapter 1	**Chapter 2**	**Chapter 3**
Original idea	KS	KS	KS, **TL**
Study design	KS, **TL**	KS, JP (2012), TC	KS, **TL**
Sampling	RK, **TL**, KS, TC	TC, KS (2012), JP (2012), DP (2013), CS (2013)	**TL**, KS
Measurements	**TL**, KS	KS (both years), JP (2012), TC, **TL**, DP (2013), CS (2013)	**TL**, KS
Data processing	**TL**, RK, KS, TC	TC, **TL**, KS, EER, JP (2012)	**TL**, KS
Manuscript writing	**TL**	TC	KS, **TL**
Finalizing submitted manuscript	**TL**	TC	KS, **TL**
Revision of manuscript for publication	**TL**	TC	KS, **TL**
Contribution to the manuscript	KS, TC, RK, HK	KS, CS, EER, JP, **TL**	No other contributions

Clarifications

TL has started his Ph.D. thesis after the experiments at Tvärminne Zoological Station (chapter 2) and the research cruise in 2013. Thus, he could not take part in the decision-making within the project so far. TL participated in the organization and execution of the other cruises (2014 – 2016). Regarding chapters 1 and 3, TL contributed significantly to the interpretation of the results (chapter 1: largely; chapter 3: equal shares with KS) and analyses (samples and data). TL contributed a large part to the study design for chapter 1 by selecting the final analyses (with RK) and introducing the identification of environmental drivers of plankton communities. Data processing was shared by all co-authors, and TL was mainly responsible for data analysis, and interpretation. TL wrote the manuscript with contributions from all co-authors and HK. Chapter 2 was also part of another dissertation (Maria Teresa Camarena-Gómez, defended in April 2019, U Helsinki). TL was responsible for 50% of the flow cytometry (bacterial abundances), >50% of the plankton microscopy, and the major part of the data preparation of the latter for further analyses. TL participated in the writing of the materials and methods section and commented on the first draft of the manuscript. KS wrote the first draft of chapter 3, and it was finalized for submission and revised for publication by KS and TL in equal shares. TL had the idea to distinguish / quantify all identifiable particles in addition to the ones considered by the Finnish plankton monitoring program. Without this extra effort, this study would not have been possible, and thus, TL was stated as lead author. KS had the idea to utilize the cruise data for this publication, when the planned papers for this thesis had to be changed.

List of abbreviations (in order of appearance)

N	Nitrogen	**CTD**	Conductivity temperature depth
P	Phosphorus	**DSi**	Dissolved silicate
C	Carbon	**EtOH**	Ethanol
BSAP	Baltic Sea Action Plan	**POC**	Particulate organic carbon
SST	Sea surface temperature	**PON**	Particulate organic nitrogen
DDP	Diatom-dinoflagellate-proportion	**POP**	Particulate organic phosphorus
DOC	Dissolved organic carbon	**BSi**	Biogenic silicate
TVZ	Tvärminne Zoological Station	**HNF**	Heterotrophic nanoflagellates
GOF	Gulf of Finland	**GPP**	Gross primary production
BCC	Bacterial community composition	**rRNA**	Ribosomal ribonucleic acid
Chl *a*	Chlorophyll *a*	**OTU**	Opertational taxonomic unit
SYKE	Finnish Environment Institute	**BA**	Bacterial abundance
FINAS	Finnish Accreditation Service	**BPT**	Bacterial production of thymidine
qPCR	quantitative polymerase chain reaction	**BPA**	Bacterial production of leucine
DinoComplex	Dinoflagellate complex	**TCA**	Trichloroacetic acid
DNA	Deoxyribonucleic acid	**FC**	Flow cytometry / cytometer
DIADOM	Diatom-derived dissolved organic matter	**E**	Stratification index
Si	Silicate	**MDC**	Microscopy derived carbon
BP	Baltic Proper	**ANOVA**	Analysis of variance
UMLD	Upper mixed layer depth	**RDA**	Redundancy analysis
BB	Bothnian Bay	**NMDS**	Non-metric multidimensional scaling
FINMARI	Finnish Marine Infrastructure	**GAM**	Generalized additive model
Dino1-2	Dinoflagellate treatments	**PERMANOVA**	Repeated measures analysis of variance
BS	Bothnian Sea	**UV**	Ultra violet
DON	Dissolved organic nitrogen		

Contents

1.) Introduction

1.1) Climate change – Recent knowledge, predictions, and effects on the Baltic Sea ecosystem

Climatic changes will affect the structure of future ecosystems around the globe (Walther et al. 2002). With the beginning of industrialism, human activities have drastically impacted the environment by burning fossil fuels and using fertilizers in agriculture, amongst others. These interventions have caused the increases of carbon dioxide (CO_2) and temperature in the Earth´s atmosphere and ocean, as well as the input of nutrients (mainly nitrogen [N] and phosphorus [P]) to natural waters. This has direct and indirect effects on aquatic ecosystems. An increase in CO_2 will acidify the sea and affect, for example, organisms with calcium carbonate structures such as mussels and coccolithophores. The latter are an essential group of marine phytoplankton, which plays a vital role in the production of the oxygen (O_2) we all breathe. An increase in water temperature will directly affect the metabolism of all kinds of organisms, since the required enzymes have temperature optima, and indirectly by drastically affecting different types of habitats. As climatic changes are already evident for aquatic habitats like the Baltic Sea, it is crucial to understand the drivers of biological processes, affecting their community composition and productivity (Andersson et al. 2015).

The presented study was carried out in the Baltic Sea, one of the world´s largest brackish water systems. It is characterized by steep horizontal and vertical gradients of salinity, O_2, and temperature (spatially and temporally). Only organisms adapted to these specific conditions form viable populations, with higher costs for maintaining their physiology compared to related marine or freshwater species (Feistel et al. 2008). Limited water exchange with the North Sea (semi-enclosed) and the high population density of the Baltic Sea catchment area (nine countries, around 85 million people) have led to several environmental problems. Due to the shallow average water depth (54 m), atmospheric forcing controls the dynamics of the Baltic Sea (Leppäranta & Myrberg 2009). Low water depth in combination with the limited water exchange, makes it vulnerable to eutrophication, decreasing water quality, loss of biodiversity, reduction of fish stocks, and acidification (Meier et al. 2012b, HELCOM 2018).

Recently, novel modeling approaches were used to study the effects of climate change on the Baltic Sea ecosystem. Several authors (e.g., Meier et al. 2012b, Andersson et al. 2015, Thomas et al. 2017) have predicted an increase in the sea surface temperature (SST) of 2-4 ºC until the end of this century. According to Meier et al. (2012b), the temperature will increase to maximum values since the beginning of weather observations (1850), whereas salinity and O_2 concentration will decrease to their lowest values ever recorded. Hypoxia / anoxia will increase the mortality of benthic organisms such as shellfish and bottom-dwelling fish (Diaz & Rosenberg 2008). With a further decrease in bottom-water O_2, the cod biomass is predicted to decrease despite reduced fishing pressure (Meier et al. 2012b), with implications for commercial fisheries. Additionally, due to top-down food web effects, a decrease in cod may lead to decreasing zooplankton abundances and thus, increasing phytoplankton biomass in the future (Casini et al. 2008).

Precipitation is supposed to increase by 30 % in the northern Baltic, resulting in increased river discharge and thus, increasing levels of allochthonous organic matter (terrestrial OM) and organic pollutants (Andersson et al. 2015). Increasing terrestrial OM levels are expected to have drastic effects on the Baltic Sea food web (see community changes below). The freshening of the Baltic will modify the distribution of many different taxa, as their boundaries are set, for example, by the wide salinity gradient (Neumann et al. 2012). An increased nutrient flow from sediments caused by increasing water temperature, combined with increased precipitation, is expected to have a substantial effect on the biogeochemistry of eutrophicated seas in the near future (Meier et al. 2012a). Another consequence of increasing

temperature is the reduction in sea-ice (BACC 2015), and the Baltic ice cover will be reduced by 50–80 % by the end of this century (Andersson et al. 2015). Furthermore, increased wind speeds are predicted and will result in an increase in wind-driven mixing of the water column, affect the flow of nutrients, and thus, the distribution of plankton assemblages (Dzierzbicka-Głowacka et al. 2011). Both spring and summer blooms of phytoplankton are suggested to increase in the future due to, for example, increases in inorganic nutrients even with the present anthropogenic loading (Meier et al. 2012a).

Along with warming and decreasing O_2 levels, internal feedback loops could be reinforced (Vahtera et al. 2007, Neumann et al. 2012, Meier et al. 2012c) and increased P-release from the sediment, reduced denitrification, and increased fixation of atmospheric nitrogen (N_2) by diazotrophic cyanobacteria, are expected. The combination of increased temperature and eutrophication causes harmful algal blooms (e.g., Maso & Garcés 2006) formed by certain dinoflagellates (e.g., *Alexandrium tamarense*) and cyanobacteria. The latter is of particular concern, since these prokaryotes may introduce ≤50 % of total N to the Baltic Sea (Wasmund et al. 2005), intensifying the eutrophication problem and affecting the recreational value of coastal areas (Andersson et al. 2015). Bloom-forming populations of the toxic dinoflagellate *Alexandrium ostenfeldii* were reported in, for example, the Åland Sea during summer (Kremp et al, 2009) but its biomass-contribution varies interannually (Jerney et al. 2019). Thus, potentially harmful dinoflagellates have already established in the Baltic Sea (Setälä et al. 2014). Decreasing diatom-dinoflagellate-proportions can be expected to have negative effects on the transfer of organic C from primary (phytoplankton) to secondary (e.g., bacteria and mesozooplankton) producers with implications for higher trophic levels such as fish. According to Suikkanen et al. (2013), mixotrophs (capable of photosynthesis and the ingestion of particles / organic molecules as sources of energy) and species considered to have a low nutritional status became more abundant during summer in the northern Baltic Sea (e.g., Gulf of Finland [GOF] and Baltic Proper [BP]).

Recent studies reported an extended phytoplankton growing season due to increasing temperatures (Hjerne et al. 2019, Wasmund et al. 2019). Wasmund et al. (2019) found that the entire phytoplankton growing season in the western Baltic Sea (at one coastal station) is prolonged, due to climate change, and presently expands from February to December (based on 29 years of data). The earlier start of the spring bloom is caused by an earlier onset of stratification (increasing temperature) in the open waters of the central Baltic Sea (Kahru et al. 2016). Besides temperature, light availability, salinity, and nutrients may affect the duration of the growing season (Wasmund et al. 2019). The longer growing season is more due to the extended autumn bloom than to the earlier onset of the spring bloom (Kahru et al. 2016, Wasmund et al. 2019). Increasing temperatures stimulate zooplankton to a more considerable extent than phytoplankton, and thus, the gap between the phytoplankton peak and the development of a microzooplankton community is reduced (Aberle et al. 2012). Therefore, elevated temperatures may cause a reduction in the overall phytoplankton biomass (Sommer & Lewandowska 2011), assuming that the predominant plankton species are edible for grazers. A phytoplankton biomass reduction may also result from efficient sedimentation of cells from the photic zone. It is important to note that the exact processes affecting phytoplankton biomass are difficult to predict. For instance, increased grazing is likely to boost the regenerated production of phototrophs, and thus, their overall annual biomass could also increase. The dominance of organisms, such as dinoflagellates, that sink less efficiently than diatoms and are thought to be lower quality food items could prevent the reduction of the phototrophic biomass. Wasmund et al. (2019) mentioned that increasing temperatures would affect different species to different extents. For example, the growth of cold-water species might be shifted to periods of colder waters (for example from summer to autumn), partly explaining the increase of the autumn biomass. Also, in this case, an increase in biomass requires that grazing and sinking is not efficient. The earlier onset of the spring bloom might be supported by a larger overwintering population arising from the higher biomass in autumn (Wasmund et al. 2019).

The results of different modeling studies regarding the effects of climate change on the Baltic Sea ecosystem differ. Neumann (2010) predicted a temperature increase of only 1 ºC, improving O_2-conditions of the deep water, and no changes in phytoplankton biomass and N-fixation rates. The predictions for different sub-basins and the outcomes of different models vary as well, and scenarios concerning the Gulf of Bothnia are considered as unreliable (Meier et al. 2011). The Baltic Sea Action Plan (BSAP) is expected to result in a distinct improvement of the environmental status, but modeling uncertainties of future scenarios (e.g., regarding salinity) complicate an accurate prediction (Saraiva et al. 2019). It is crucial to resolve the difficulties of predictive modeling of the changing ocean by, for example, considering empirical data from important seasons like the spring bloom in temperate areas (e.g., presented studies).

It is urgent to assess the impacts of the combined effects of climate change and future human activities on marine ecosystems like the Baltic Sea. The current government policies are not sufficient to improve the water quality of the Baltic Sea until the year 2100, and due to climatic changes, eutrophication is likely to increase even further (Meier et al. 2012a). The BSAP aims to reduce nutrient loads to the Baltic (HELCOM 2007), but future changes in climatic conditions were not considered (Meier et al. 2012c). However, human activities are thought to supersede the effects of climate change on nutrient loading (Pihlainen et al. 2020). Unfortunately, stakeholders do not adequately consider climate change as a current issue for the Baltic and adaptation and mitigation strategies find little attention (Meier et al. 2012b). Still, currently there are some efforts to consider climate change for the protection of the Baltic Sea (details on http://smartsea.fmi.fi/). Thus, it is even more crucial to raise public awareness on the possible impacts of climate change on marine ecosystems, to protect the ocean, including the Baltic Sea, as an essential food source and its pivotal role in, for example, the C-cycle and thus, the global climate. Only a reduction in nutrient loads can aid the regeneration of the Baltic ecosystem in the long run (Gustafsson 2008, Conley et al. 2009), and everybody can contribute to this. In the future, managing programs should consider the confounding effects of climatic changes, eutrophication, and pollution on ecosystem-functioning (Andersson et al. 2015).

1.2) The spring bloom in the Baltic Sea – Characteristics and important plankton species / groups

In the Baltic Sea, spring is by far the most productive period of the year. Approximately 50 % of the annual C-fixation occur in only about one month (Lignell et al. 1993, Heiskanen 1998). As in most temperate regions, the Baltic spring bloom is triggered by increasing light availability after the winter (e.g., Neumann et al. 2012). The phytoplankton community is dominated by nano- and microplankton (2-20 and 20-200 µm) and the co-occurrence of diatoms and dinoflagellates is characteristic for the Baltic Sea (Wasmund & Uhlig 2003, Kremp et al. 2008). During the bloom, five diatoms (*Thalassiosira baltica*, *T. levanderi*, *Achnanthes taeniata*, *Skeletonema marinoi*, and *Chaetoceros* spp.) and three phototrophic dinoflagellates (*Peridiniella catenata*, *Gymnodinium corollarium*, and *Biecheleria baltica*) may contribute significant biomass-proportions and thrive in different bloom phases (Höglander et al. 2004, Legrand et al. 2015, Spilling et al. 2018). Due to the high sedimentation rates during the bloom (Heiskanen 1998), the amount of detritus in the surface water is usually constant and low during the spring bloom (Lipsewers & Spilling 2018). Towards the end of the bloom and at post-bloom conditions (May / June), certain organisms like the mixotrophic ciliate *Mesodinium rubrum*, an important primary producer at this phase (Lips & Lips 2017), become more abundant. Beside *M. rubrum*, also certain dinoflagellates benefit from vertical migration and efficient nutrient uptake at this bloom phase (Heiskanen 1995, Lips & Lips 2017). Furthermore, heterotrophic ciliates become more relevant as the temperature increases (Mironova et al. 2012). These organisms are motile, and their vertical migration allows them

to reach food items (such as phytoplankton / bacteria and / or organic molecules) being trapped in specific water layers due to water column stratification.

G. corollarium and *B. baltica* are usually summarized to a dinoflagellate-complex (monitoring programs and presented chapters), including at least one more species (*Apocalathium malmogiense*, formerly called *Scrippsiella hangoei*). These phototrophs are indistinguishable by traditional microscopy of Lugol-preserved samples, and thus, species-specific abundances remain unknown. However, they represent three different dinoflagellate orders and differ in terms of life cycle strategies and nutrient uptake efficiencies (Sundström et al. 2009, Warns et al. 2012). Thus, valuable pieces of information are lost if they are summarized into one group. The findings of a pilot study carried out to unravel the species-specific biomass-contributions and distribution patterns of the three dinoflagellates, are presented in the results chapter of this summary. Due to different ecological strategies, changing communities (for example the prevailing dinoflagellate species) will affect the nutrients exported to the sediment and the ones available for phytoplankton growth, with implications for biogeochemical cycles (e.g., Spilling et al. 2018).

1.3) Environmental drivers and variability of the spring bloom community structure

Temperature is an essential driver of the phytoplankton community composition in the Baltic Sea. The spring community comprises several arctic marine species, such as the diatoms *Melosira arctica* and *Achnanthes taeniata* (Niemi 1973 and references therein), which may occur at high abundances in and below the sea ice, respectively (Haecky et al. 1998). The dinoflagellate *Peridiniella catenata* is classified as an Arctic-boreal species and may occur in high abundances inside sea-ice as well (Okolodkov 1999). Due to its tolerance to a wide salinity-range and low temperature, this species can be found in, for example, the Baltic Sea and the Fram Strait, which is located between Iceland and Greenland (Okolodkov 1999). Elevated temperatures are expected to have drastic effects on the abundances of such cold water-adapted species and might even cause their extinction. The concentration of inorganic nutrients in surface waters is known to be another main driver of the community structure (Margalef 1978, Glibert 2016). Also, different upper mixed layer depths (UMLD, affected by temperature) favor different organisms. These environmental factors determine the succession of different taxa and the phytoplankton biomass, measured as Chlorophyll *a* (Chl *a*), along with the spring bloom in the Baltic Sea (Fig. 1, based on presented data). The temperature increase will intensify the stratification of the water column (HELCOM 2013b), and thus, motile organisms will benefit (Heiskanen 1995). Additionally, increased stratification (shallower mixed layer) will cause a decrease in nutrient supplies to the upper mixed layer, favoring the growth of smaller taxa (Pasciak & Gavis 1974, Irwin & Oliver 2009).

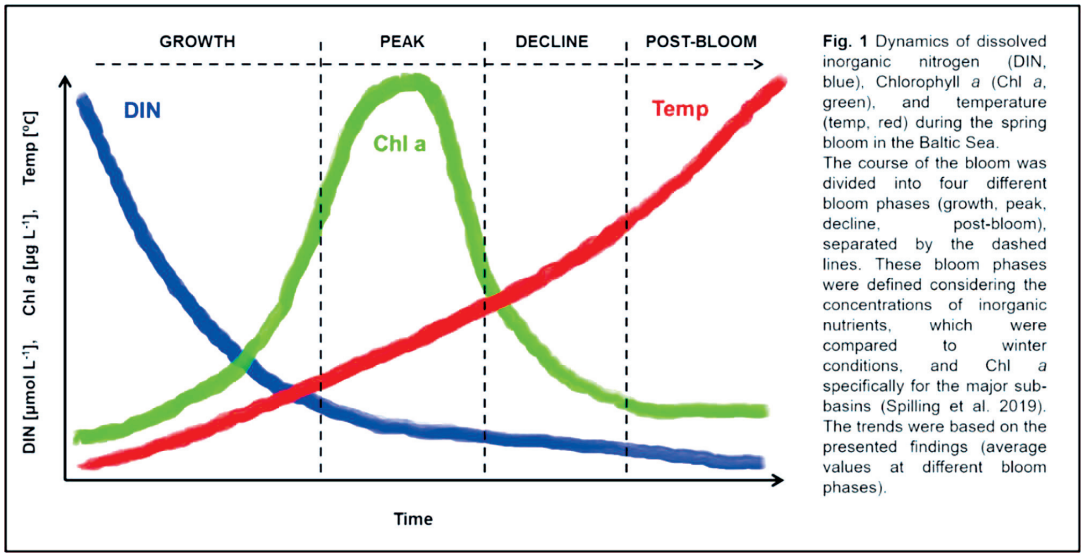


Fig. 1 Dynamics of dissolved inorganic nitrogen (DIN, blue), Chlorophyll *a* (Chl *a*, green), and temperature (temp, red) during the spring bloom in the Baltic Sea. The course of the bloom was divided into four different bloom phases (growth, peak, decline, post-bloom), separated by the dashed lines. These bloom phases were defined considering the concentrations of inorganic nutrients, which were compared to winter conditions, and Chl *a* specifically for the major sub-basins (Spilling et al. 2019). The trends were based on the presented findings (average values at different bloom phases).

The Baltic spring species are cold water-adapted, and community shifts due to direct / indirect effects of global warming have been observed (Wasmund & Uhlig 2003, Klais et al. 2011, Klais et al. 2013). Changing climatic conditions, such as a reduction in sea-ice and increased wind speeds, have modified mixing regimes and caused a decrease in the diatom-dinoflagellate-proportion (DDP) during spring in several sub-basins (Klais et al. 2013). Furthermore, inter-annual differences in pre-bloom conditions result in different dominance patterns during the actual bloom (Legrand et al. 2015). Generally, mild winters (thin or absent ice-cover) and increased wind speeds favor (dino)flagellates, whereas diatoms thrive after cold winters (thick ice-cover). Diatoms prefer mixed / cold waters (Finkel et al. 2010), and as winters will become milder, the overall diatom-biomass is expected to decrease further.

Diatoms play a vital role in the Baltic Sea food web (Wasmund et al. 2011), and the annual phytoplankton biomass is not compensating the lower contribution of diatoms during the spring bloom after mild winters (Legrand et al. 2015). This is expected to cause a reduction in the primary production utilized by secondary producers in the future (Legrand et al. 2015). Since diatoms sink to the seafloor rapidly after nutrients (mainly N) are limiting / depleted in the photic zone (e.g., von Bodungen et al. 1981), they play a significant role for the benthic-pelagic coupling (Heiskanen 1998). The biomass-relevant dinoflagellates in the Baltic form resting stages (Kremp et al. 2018), which are less bioavailable for other organisms. Thus, these particles may function as a “long”-term sink for inorganic nutrients and C. Depending on the dominant species, dinoflagellates might have beneficial effects on the ecosystem by contributing to a reduction of pelagic nutrients. An increase in smaller taxa (see above) is also valid for the Baltic Sea, where size classes <10 µm become more important for primary production at late bloom phases with elevated temperatures and low nutrient concentrations (Spilling et al. 2019). Thus, phytoplankton community shifts may have far-reaching consequences for the transfer of energy to higher trophic levels (Vehmaa et al. 2011) and the biogeochemical cycling of nutrients (Höglander et al. 2004, Spilling et al. 2018).

Furthermore, community changes will likely influence both ecological interactions in the pelagic food web (changing the conditions for competitors and / or grazers) and the amount / quality of bioavailable OM, sinking out of the photic zone (Tamelander & Heiskanen 2004). The bioavailable OM is an energy source for heterotrophic organisms, such as benthic invertebrates and heterotrophic bacteria. In contrast, living phytoplankton serves as an important food source for zooplankton, such as copepods. In the Baltic spring, phytoplankton is more bottom-up controlled (by light, nutrients, and temperature) before the development of a mesozooplankton community. Changes in the phytoplankton community structure might

have direct bottom-up effects on herbivorous zooplankton later during the season (De Bernardi & Giussani 1990, Sopanen et al. 2008).

The community composition and magnitude of the spring bloom determines the pelagic P-concentration after inorganic sources of N have been exhausted, which has implications for the development of, for example, toxic cyanobacteria in the summer. Shifts in the phytoplankton spring community might alter the concentration of particulate organic nutrients, increasing the amount of C relative to growth-limiting nutrients in the sinking biomass, and thus, affect levels and ratios of pelagic nutrients available for phototrophic growth. Unlike the P-pool (internal feedback loop), the Baltic spring bloom usually depletes the inorganic N-pool in most sub-basins (Tamminen & Andersen 2007). However, assuming there is remineralized N available, the environmental N:P ratios at post-bloom conditions will be very low. Certain dinoflagellates feature lower inorganic N:P requirements compared to diatoms (Shi et al. 2005), and thus, may win the competition at low N:P supply ratios (Hodgkiss & Ho 1997). These conditions are characteristic for later bloom phases, for example, in the commonly N-limited GOF (Tamminen & Andersen 2007), which is dominated by vertically migrating dinoflagellates at this time of the year (Heiskanen 1995, Lips et al. 2014). The increases in N and P, are variably proportional to the C input, which may cause drastic changes to biogeochemical cycles (e.g., Falkowski et al. 2000) and could affect the C:N:P ratio in the phytoplankton biomass amongst others. Variations in the elemental composition of prey organisms may affect zooplankton growth, food web dynamics, and the remineralization of nutrients, with implications for the global C-cycle, playing a crucial role in climate change (Sterner & Elser 2002).

The projected increase in allochthonous OM, triggered by increased precipitation (Andersson et al. 2015), corresponds to a decrease in light availability. These conditions might favor the growth of heterotrophic bacteria at the expense of phytoplankton (Sandberg et al. 2004), and additionally open new niches for non-native species (Andersson et al. 2015). It is expected that the structure of the food web in the northern Baltic (especially Bothnian Bay, BB) is gradually changing to a system where more energy is flowing through the microbial loop, as outlined in the following section (Andersson et al. 2015).

1.4) Changes in the microbial loop during spring and consequences for the energy transfer within the Baltic Sea food web

In terms of OM export (quantity and quality) to the seafloor, it makes a clear difference, which group / species dominates the phytoplankton spring bloom. As mentioned before, diatoms are known to sink out of the photic zone rapidly after the depletion of N (von Bodungen et al. 1981, Heiskanen 1998). Dinoflagellates either disintegrate in the water column (Heiskanen 1998) or form resting stages. These cell types may not be bioavailable for heterotrophic organisms (e.g., Spilling & Lindström 2008). Thus, diatoms are probably a higher quality energy-source for heterotrophic bacteria, compared to dinoflagellates (Heiskanen 1998, Höglander et al. 2004). The release of dissolved organic carbon (DOC) is highly species-specific (Wetz & Wheeler 2007), and certain dinoflagellates excrete more DOC than diatoms (Castillo et al. 2010, López-Sandoval et al. 2013). Spilling et al. (2014) found that DOC-excretion is higher when diatoms dominate the bloom in the Baltic Sea. Due to the different origins and release-processes of dissolved organic matter (active excretion, viral lysis, sloppy feeding by grazers, and cell degradation), the pool of dissolved organic matter (DOM) is highly diverse. It comprises a labile (bioavailable) and a more refractory (not bioavailable) fraction (Amon & Benner 1996). Carbohydrates, proteins, and amino acids are the types of DOM mostly excreted by diatoms (Myklestad 1995, 2000, Urbani et al. 2005, Thornton 2014), whereas fatty acids and lipids are also excreted by dinoflagellates (Parrish et al. 1994). The labile DOM fraction is rapidly taken up by microorganisms, partly transferred to higher trophic levels via the microbial loop, or respired to a certain extent (Azam et al. 1983, Smith et al.

1995). The increase of the SST, and thus, climate change-related stress, is suggested to elevate the DOM-excretion by phytoplankton (Thornton 2014). As a result, the amount of DOC entering the microbial loop would increase. If more energy (organic C) is channeled through the microbial loop, higher abundances of heterotrophic bacteria can be expected, most likely resulting in higher abundances of heterotrophic ciliates, an essential group of microzooplankton (Calbet et al. 2019). Higher abundance of heterotrophic bacteria would enhance the competition for inorganic nutrients between bacteria and phytoplankton as well as the remineralization by, for instance, heterotrophic ciliates in the surface water (Lips 2020, personal communication).

As the DOM-release by phytoplankton is species-specific, the dominant species determines the quantity and quality of the DOM pool available for the bacterial community. Different types of DOM represent different niches for bacteria, affecting their growth and community structure (Biddanda & Benner 1997, Riemann et al. 2000, Buchan et al. 2014). For instance, Alphaproteobacteria are more abundant before the phytoplankton spring bloom (pre-bloom phase), whereas Gammaproteobacteria, Bacteroidetes, and Flavobacteria are more relevant during the bloom and / or the post-bloom phase (Cottrell & Kirchman 2000, Pinhassi et al. 2004, Teeling et al. 2012, Laas et al. 2015, Bunse et al. 2016). The most important genera of the latter two classes are *Flavobacterium* and *Pseudomonas*, which are closely linked to diatom blooms (Amin et al. 2012). The less abundant Actinobacteria and Betaproteobacteria are commonly found in the Baltic Sea during or after the spring bloom (Riemann et al. 2008, Herlemann et al. 2011, Bunse et al. 2016). Furthermore, environmental factors affect the community compositions of both the phytoplankton and the bacterioplankton. For instance, an increase in temperature is known to boost the bacterial production (Hoppe et al. 2008, von Scheibner et al. 2014). The activity of a phytoplankton cell (different growth phases) defines the excretion of excess organic C (Biddanda & Benner 1997, Meon & Kirchman 2001, Thornton 2014). Thus, the bloom phase has a strong effect on the bacterial community. According to Camarena-Gómez et al. (2018), the shift towards more dinoflagellate dominated blooms is likely to decrease bacterial production and alter the bacterial community composition. In turn, this could lead to a reduction in pelagic OM remineralization and modified C-fluxes within the microbial loop. A less efficient channeling of organic C to higher trophic levels via the microbial loop, compared to the classical food chain (Suikkanen et al. 2013), could have severe effects on fish-stocks (Andersson et al. 2015), and thus, on top predators such as birds and seals.

1.5) Microzooplankton – Composition and role in the microbial food web

Microzooplankton comprises heterotrophic and mixotrophic organisms, ranging from 20 to 200 µm in size. This group consists of phagotrophic protists, dinoflagellates, ciliates, acantharids, radiolarians, foraminiferans, and juveniles of mesozooplankton, amongst others (Calbet et al. 2019). Thus, it is a heterogeneous group with a common feature, the capability to engulf particles for energy and nutrient uptake, which is referred to as phagotrophy.

Currently, microzooplankton receives far less attention in monitoring programs across the Baltic Sea than phyto- and mesozooplankton, and they have been mostly neglected in studies on the spring bloom. This is unfortunate, as the role of microzooplankton in the aquatic food web is particularly important in linking the microbial loop with higher trophic levels (Calbet & Landry 2004), and it is crucial to follow the future dynamics of these organisms in changing marine ecosystems (Calbet 2008). Furthermore, heterotrophic ciliates can clearly reduce the abundances of bacteria and phytoplankton, especially at elevated temperatures (Mironova et al. 2012). According to Calbet et al. (2019), microzooplankton grazing consumes approximately 60 % (on average) of the primary production in temperate waters, equivalent to more than half of the phytoplankton biomass per day. These organisms are also important recyclers of inorganic nutrients. Calbet et al. (2019) estimated that the microzooplankton

recycles between 40 and >60 % of the N required for phototrophic growth, which is clearly more than the contribution of the larger copepods (14 %). The Baltic spring bloom is dominated by phytoplankton and the grazing pressure by mesozooplankton is limited (Lignell et al. 1993). Therefore, it can be expected that the share of unaccounted biomass is even higher during summer with higher contributions of microzooplankton (Mironova et al. 2012). Along with higher heterotrophic activity and bacterial production, an increase in the spring-biomass of heterotrophic ciliates can be expected at elevated temperatures. Heterotrophic ciliates are a crucial part of the microbial loop (Caron et al. 1982, Fuhrman & Caron 2016) and the regenerated production and top-down control of the phytoplankton biomass will likely become more relevant (Hoegh-Guldberg & Bruno 2010). In turn, smaller heterotrophic groups (such as microzooplankton) could become more important, and cause temporal and spatial shifts of zooplankton populations (Calbet 2008, Richardson 2008), affecting higher trophic levels.

Food web dynamics are also affected by higher levels of allochthonous OM in the sea, caused by increased river-discharge. Increased concentrations of this terrestrial OM will favor heterotrophic bacteria (Andersson et al. 2015), because it is an additional source of organic C, besides phytoplankton-derived DOC. Thus, higher contributions of heterotrophs (e.g., bacteria and grazing ciliates) will alter the type of C (energy)-source used by planktonic organisms. A net heterotrophic community uses more organic C for respiration and thereby produces CO_2, whereas a predominant phototrophic community uses inorganic C (CO_2) during photosynthesis and produces O_2. An increase of heterotrophs at the expense of phototrophs might result in an increase in net respiration of the plankton community and a less permanent burial of CO_2 in sediments. Approximately 50 % of the O_2 in the Earth´s atmosphere originates from the aquatic community of phototrophs, which is dominated by phytoplankton (Falkowski & Raven 1997). Thus, the plankton community composition directly affects the atmospheric composition of gases, and thus, the global climate. As a net phototrophic community provides the atmosphere with O_2 (positive effect), an increase in net respiration might negatively affect the atmospheric composition of gases, contributing to the overall expansion of CO_2 in the atmosphere and the surface ocean, and the resulting climatic changes.

1.6) Environmental monitoring and its scientific use

Environmental monitoring is an essential tool to assess the current status of ecosystems, such as the Baltic Sea and follow the ongoing changes in ecosystem functioning. Monitoring programs strive to consider most recent technical and conceptual advances to produce meaningful datasets and study, for instance, the long-term development of an ecosystem and the effects of changing environmental conditions. Several plankton groups (microzooplankton, nano- / picoplankton, and heterotrophic bacteria) are suggested to become more abundant within the scope of climate change, and it will be a challenge for monitoring programs to register these modifications in community composition. Phytoplankton forms the base of aquatic food webs, and negative climatic effects on their base will also affect higher trophic levels (e.g., Andersson et al. 2015). The plankton community composition affects the biogeochemistry of the ocean and modeling studies considering the different functional groups can contribute significantly to understand marine material fluxes (Litchman et al. 2015, Vichi et al. 2015). Time series data can be used to evaluate the ecosystem structure and functioning, i.e. community composition and food web assessment (Wasmund et al. 2011, Lehtinen et al. 2016, Klais et al. 2017). Monitoring programs may also form the basis for decisions to change management practices to maintain or improve the environmental conditions (Borja et al. 2016). Data harmonization and publicly available datasets are required to enable and promote the scientific use and increase the value of monitoring data (Klais et al. 2015, Zingone et al. 2015).

2.) Aims of the presented studies

The presented thesis was motivated by the need to understand the causes and particularly consequences of the recently observed shift in the phytoplankton community composition during the Baltic Sea spring bloom. In the last decades, the contribution of dinoflagellates to the total biomass increased relative to diatoms in several sub-basins, which was expected to have ecosystem-wide consequences. These changes in community structure were caused by climatic changes such as a decrease in sea ice and increasing wind speeds. The main aim of the presented study was to investigate the effects of these changes at the base of the food web during the Baltic Sea spring bloom, with emphasis on the connections between environmental variables and the nano- / microplankton community composition. Furthermore, links between different communities and the elemental composition (stoichiometry) of the seston (the sum of all particles suspended in natural waters), responses (metabolic activity) of heterotrophic bacteria, and contributions of microzooplankton during the early growing season, were studied.

The entire research for this thesis was carried out in the Baltic Sea at spring bloom conditions. Specifically, this study aimed to answer the following research questions:

1) What are the environmental factors affecting the nano- and microplankton community structure?
2) Is the seston nutrient stoichiometry affected by the community structure, and if yes, are these effects predictable for the spring season?
3) What is the role of microzooplankton in spring, and what does this mean for food web dynamics and future monitoring programs?
4) How do heterotrophic bacteria react to different dominance patterns of diatoms and dinoflagellates, and thus, various sources of labile DOM, and what are the consequences for the microbial loop?

Answering questions 1 to 3 was targeted with the aid of research cruises (chapters 1 and 3) and question 4 experimentally (chapter 2).

3.) Material and methods

3.1) Study design

All studies presented in this thesis were related to the phytoplankton spring bloom in the Baltic Sea. Surface water samples (z = 3 m) were collected during different phases (April / May) of four subsequent spring blooms aboard R/V Aranda (2013-2016), with the Gulf of Finland (GOF) and Baltic Proper (BP) being the most frequently sampled sub-basins (Fig. 2A). An extensive spatial coverage of the Baltic Sea (7 sub-basins, 119 stations considered for chapter 1, 125-127 for chapter 3) was achieved by combining the four cruises. The resulting dataset was used for chapter 1 (Lipsewers et al. 2020, in press) and 3 (Lipsewers & Spilling 2018). Chapter 2 (Camarena-Gómez et al. 2018) was based on two experiments carried out at Tvärminne Zoological Station (TVZ, University of Helsinki) on the south-west coast of Finland (also GOF). The water for these experiments was collected in the proximity of TVZ (z = 20 m) when the sea was still ice-covered (February / March). For both experiments, the natural water (no community manipulations) served as a control treatment. Different strains (monoalgal cultures) of diatoms and dinoflagellates were added to the natural pre-bloom communities (low biomass, high nutrient concentrations) to achieve different dominance patterns of certain species (different experimental units). These strains were provided by the FINMARI (Finnish Marine Infrastructure) culture collection, originally isolated from the Baltic

Sea, and they can individually dominate the spring bloom. In 2012, the treatments were Diatom1 (*Chaetoceros wighamii* and *Thalassiosira baltica*) and Dino1 (DinoComplex [*Gymnodinium corollarium*, *Biecheleria baltica*, *Apocalathium malmogiense*] and *Peridiniella catenata*) and in 2013, Diatom2 (*T. baltica*), Diatom3 (*Achnanthes taeniata*), and Dino2 (*B. baltica*). In both years, the temperature was increased from 4 to 10 ºC (day 19) to boost bacterial growth after the depletion of inorganic nutrients.

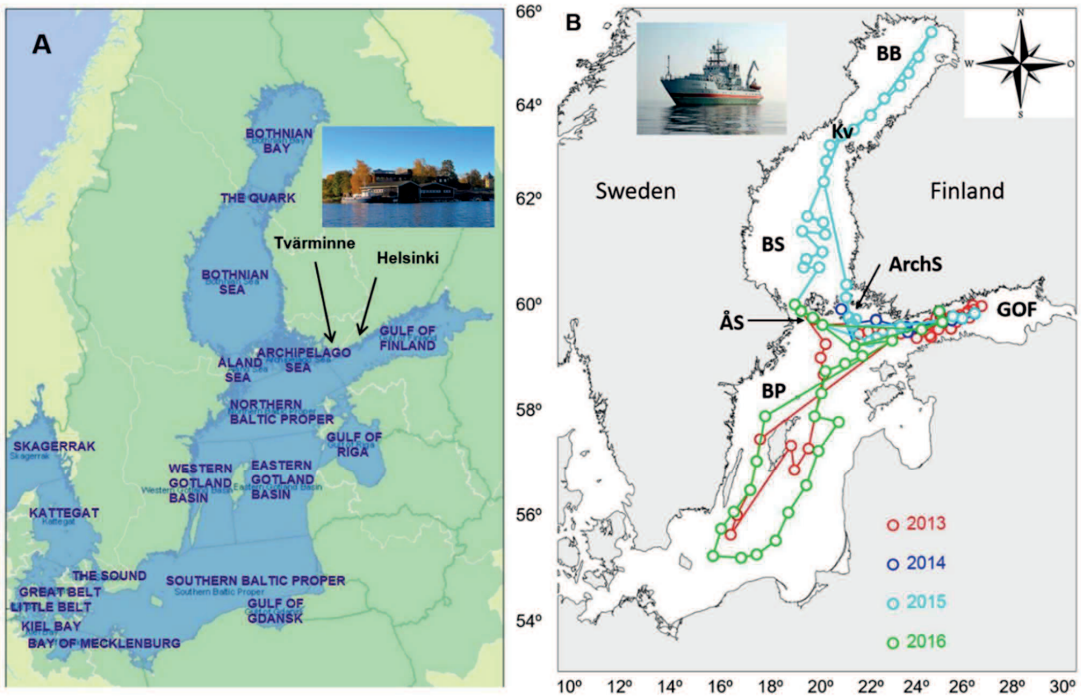


Fig. 2 A: The Baltic Sea and its sub-basins (HELCOM, modified after Gollasch et al. 2011). The locations of Tvärminne Zoological Station (Tvärminne, picture by Joanna Norkko) and the start and end of the four cruises (Helsinki) are indicated by the arrows. B: The sailing routes of the cruises aboard R/V Aranda in different colors (figure by Riina Klais, picture by Jan-Erik Bruun). Each circle represents a sampling station. BB = Bothnian Bay, Kv = Kvarken (Quark), BS = Bothnian Sea, ArchS = Archipelago Sea, ÅS = Åland Sea, GOF = Gulf of Finland, BP = Baltic Proper.

Fig. 2A shows the Baltic Sea with its sub-basins, including the ones sampled during the cruises (modified after Gollasch et al. 2011). Additionally, the location of Tvärminne (experiments for chapter 2) and Helsinki (start and end of the cruises) are indicated. The Quark is a narrow strait between the Bothnian Sea (BS) and the BB, and together, these sub-basins form the Gulf of Bothnia. The sailing routes and sampling stations of the research cruises are depicted in Fig. 2B.

3.2) Summary of material and methods and details on specific procedures

The methods applied in the different chapters are summarized in Table 1, and protocols specific for the study area and sampling season are described in more detail. Further details on the materials and methods, such as the experimental procedure for chapter 2, can be found in the individual chapters of this thesis. Additionally, an unpublished study on dinoflagellate identification using two different methods (epifluorescence microscopy and a molecular approach) will be described. More details and ideas for improvement of the molecular tool can be found in the supplementary materials. The findings obtained so far are shown in the results section.

Table 1 Summary of the methods used for chapters 1 to 3. More details on chapters 2 and 3 are published (Camarena-Gómez et al. 2018, Lipsewers & Spilling 2018) or described in chapter 1 of the full thesis (Lipsewers et al. 2020, in press). Superscript numbers were used to match methods with the corresponding references. The determination of variables indicated with ***** involve a specific procedure for samples from the Baltic Sea and its spring season (more details below). Ch. = chapter, SYKE MRC = Finnish Environmental Institute Marine Research Centre, FINAS = Finnish Accreditation Service, Temp. = temperature, Sal. = salinity, NO_2^- = nitrite, NO_3^- = nitrate, NH_4^+ = ammonium, PO_4^{3-} = phosphate, DSi = dissolved silicate. CTD = conductivity temperature depth, UMLD = upper mixed layer depth, Chl *a* = Chlorophyll *a*, EtOH = Ethanol, POC = particulate organic carbon, PON = PO nitrogen, POP = PO phosphorus, BSi = biogenic silicate, v/v = volume/volume, DDP = diatom-dinoflagellate-proportion, HNF = heterotrophic nanoflagellates, GPP = gross primary production, HCO_3^- = bicarbonate, BCC = bacterial community composition, PCR = polymerase chain reaction, DNA = deoxyribonucleic acid, rRNA = ribosomal ribonucleic acid, OTU = operational taxonomic unit, BA = bacterial abundance, BPT = estimation of bacterial thymidine production (DNA-synthesis proxy), BPL = estimation of bacterial leucine production (protein-synthesis proxy), fin. conc. = final concentration, TCA = tri-chloro acetic acid, DOC = dissolved organic carbon, DON = DO nitrogen, acc. = according.

Ch.	Variable	Purpose	Method	References
ENVIRONMENTAL (ABIOTIC) VARIABLES				
1, 2	NO_2^-, NO_3^-, NH_4^+, PO_4^{3-}, DSi	Concentrations of dissolved inorganic nutrients. Used to define different bloom phases, study environmental drivers of the community composition, and for correlations with multiple variables	Standard colorimetric methods; Analysis by spectrophotometry or an automated analyzer	Measurements according to SYKE MRC in-house protocol (accredited by FINAS), also valid for Chl *a* and inverted microscopy Koistinen et al. (2020a) for N-species Koistinen et al. (2020b) for PO_4^{3-} Koistinen et al. (2020c) for DSi and references therein
1, 2	Temp., Sal., wind speed, bottom depth (depth profiles of, for example, density)	Studying abiotic drivers of the community composition and test links to different variables	CTD-rosette sampler, weather station, aboard facilities (Ch. 1); hand-held probes for Temp. and Sal. (Ch. 2)	SYKE MRC (Ch. 1)
1	UMLD*	Detection of thermo- and halocline (temporary and more stable UMLD´s) as indicators of pelagic stratification (e.g., light climate) and test their effects on the plankton community	Calculation based on stratification index E[1], considering the bottom depth[2] and the density differences between additive water layers[3]	Heiskanen and Kononen (1994)[1] Kuosa et al. (2017)[2] Considered individually for this study[3]
BLOOM PHASES				
1	Growth, peak, decline, post-bloom*	Studying the differences in biotic and abiotic variables between the different phases of the spring bloom	Definition of bloom phases specifically for this dataset; based on inorganic nutrients and Chl *a*	Spilling et al. (2019)
BIOTIC VARIABLES				
all	Chl *a**	To estimate the phytoplankton biomass, define bloom-phases, and calculate the Chl *a*:POC ratio (e.g., indicator for net heterotrophic / phototrophic communities)	Filtration[1]; Extraction with EtOH (96 % v/v, -20 ºC)[1]; Spectrophotometry[2]	HELCOM (2017)[1] Jespersen & Cristoffersen (1987)[2]
all	Nano- and micro-plankton (<100 µm) incl. grazers (mostly HNF and heterotrophic ciliates)*	Determining the community composition and abundances / C-biomass of up to 49 taxonomic units; Studying the effects of different plankton communities on heterotrophic bacteria and seston stoichiometry	Sample preparation[1]; Inverted microscopy of Lugol-preserved samples (different counting strategies for field studies[2a] and experiments[2b]); Species identification[3] (details below); biovolumes[4]; C-biomass[5]; DDP[6]	Utermöhl (1958)[1] SYKE MRC in-house protocol[2a] and own strategy[2b] Hällfors & Hällfors (2003) and Hällfors (2004)[3] Olenina (2006)[4] HELCOM (2013)[4] Menden-Deuer and Lessard (2000)[5]

				Auf dem Venne (1994)[5] Putt and Stoecker (1989)[5] Wasmund et al. (2017)[6]
1, 2	GPP*	Studying the effects of different communities and bloom phases on the gross primary (phototrophic) production	Based on uptake of ^{14}C-labeled dissolved HCO_3^- by phototrophs[1]; A specific procedure for Baltic Sea samples[2] and spring samples[3] was followed	Steeman-Nielsen (1952)[1] Gargas (1975)[2] Spilling et al. (2019)[3]
2	BCC	Studying the bacterial community composition mainly concerning different phytoplankton communities, bloom phases	DNA-extraction (filters)[1]; 2-step PCR to amplify the target region (V1 to V3 of the 16S rRNA)[2] before Illumina MiSeq paired-end multiplex sequencing	Power Soil DNA isolation kit (Mo Bio Laboratories)[1] Primers by Chung et al. (2004) and Edwards et al. (1989)[2]
2	Bioinformatics (part of BCC analyses)	Required after sequencing of the BCC samples (above) before further analyses of final OTU´s	Primer removal[1]; Paired-end merging[2]; Quality filtration and chimera checking[3]; OTU clustering[4]; Taxonomy of OTU´s[5]; Normalization of libraries after removal of chloroplasts, mitochondria and singletons[6]	Martin (2011)[1] Zhang et al. (2013)[2] Edgar et al. (2011)[3] Edgar (2013)[4] Schloss et al. (2009) and Quast et al. (2012)[5] Paulson et al. (2013)[6]
2	BA	Abundances of heterotrophic pelagic bacteria	Samples preserved with paraformaldehyde; Flow cytometry of SYBR Green 1 stained cells	modified after Gasol and Del Giorgio (2000)
2	BPT*	Determination of bacterial thymidine (T) production to study the effects of different phytoplankton communities and bloom phases on DNA-synthesis	Based on the uptake of radiolabeled methyl-^{3}H-T (fin. conc.14-20 nM); Removal of unincorporated radioisotope (TCA method in 2012[1], centrifugation in 2013, same for BPL); Conversion of T-production to C (µmol C L^{-1} h^{-1})[3]	Fuhrman and Azam (1982)[1] Smith and Azam (1992)[2] HELCOM 2008 and Norland (1993)[3]
2	BPL*	Determination of bacterial leucine (L) production to study the effects of different phytoplankton communities and bloom phases on protein-synthesis	Based on uptake of radiolabeled 14C[U]-L (fin. conc. 100-166 nM); Conversion of L-production to C[1]	Simon and Azam (1989)[1]
PARTICULATE & DISSOLVED ORGANIC NUTRIENTS (BIOTIC ORIGIN)				
all	POC[1] and PON[2]	Concentrations of particulate organic nutrients to study, for example, the elemental composition of the seston in different communities / bloom phases. POC is also a proxy for the total plankton biomass.	Samples were filtered onto acid-washed & pre-combusted GF/F filters using glass funnels; Simultaneous detection of POC/N with the aid of a CN (element) analyzer (autosampler) or a mass spectrometer	Koistinen et al. (2020d)[1] Koistinen et al. (2020a)[2] and references therein
all	POP	Likewise.	Likewise, for samples; Colorimetric analysis by spectrophotometry or with automatic analyzer	Koistinen et al. (2020b) and references therein
all	BSi	Likewise. BSi is also a proxy for the diatom-biomass.	Samples were filtered onto polycarbonate filters using plastic funnels; wet-alkaline digestion to convert BSi to silicic acid;	Koistinen et al. (2020c) and references therein

			Spectrophotometry	
2	DOC[1] and DON[2]	Concentrations of dissolved organic nutrients (mainly excreted by phyto-plankton); Studying the effects of different DOC/N-levels (phytoplankton communities) on heterotrophic bacteria	Filters were analyzed by high-Temp. catalytic oxidation method; Carbon analyzer with autosampler and instrument-specific carrier gas and oxidizing agents; DON was only measured in 2013	Koistinen et al. (2020d)[1] Koistinen et al. (2020a)[2] and references therein

Five of the stations considered for chapter 3 were sampled from the flow-through system (aboard R/V Aranda) and most of the cruise samples were taken with the CTD (conductivity temperature depth) rosette sampler using Niskin bottles. The protocols used to determine the concentrations of dissolved inorganic nutrients and Chl *a* as well as the counting strategy used for the microscopy in chapter 1 were developed in the SYKE Marine Research Laboratory, which is accredited by the Finnish Accreditation Service (FINAS). Nutrient analyses were done by the water chemistry team (SYKE) aboard R/V Aranda and the TVZ staff, which analyzed the samples for particulate organic nutrients (all) and Chl *a* (Chapter 2).

Samples for the bacterial community composition (BCC) were taken on day 0, the Chl *a* peak, and the last day of the experiment (2012) or the bacterial production peak (2013) instead of the latter. In 2012, samples from replicate mesocosms (treatments) were pooled to determine the BCC. In 2013, all experimental units were individually considered. Thus, it was decided to apply statistical analyses for the 2013 samples, exclusively. The sequencing (and previous PCR [polymerase chain reaction], Table 1) was done by the Institute of Biotechnology at the University of Helsinki (Finland). In 2012 and 2013, different flow cytometers (FC) and thus, different measuring protocols (quality control, FC-settings, flow rate, *etc*.) and software for data analyses were used (details in chapter 2). In 2013, the samples were diluted (1:10, TE-buffer, pH 8) before analysis. Quality control (cleaning of FC-tubing, bead counts, and blank measurements) was emphasized during all FC-analyses.

Several procedures mentioned in Table 1 were considered, because they were specifically developed for the Baltic Sea (at least specific steps) and / or optimized for the Baltic Sea spring season. The specifics of these methods will be explained in the following.

Determination of the upper mixed layer depth (UMLD)

The determination of the UMLD was tailor-made for the sampled season and the Baltic Sea. Depth profiles (temperature, conductivity, salinity, Chl *a*, and oxygen) provided by the CTD-lab (R/V Aranda) were studied, and the stratification index E (Table 1) was calculated considering the density profiles for each station. Since stratification is not well pronounced in the (northern) Baltic in spring, E was calculated in 5 m increments for the whole water column to detect a change of ≥0.9 between two water layers. If this change occurred between 10 and 15 m, 12.5 m was considered as the UMLD. Stations with a linear depth profiles were found as deeply mixed (UMLD = bottom depth). As thermoclines above 20 m are mostly short-term during the Baltic spring, the more stable halocline was likewise determined for stations with low UMLD´s (<13 m). The effects of both UMLD´s on the plankton community structure were identical, and thus, only the thermocline was finally considered for the redundancy analysis (RDA).

Definition of different bloom phases

The concentrations of dissolved inorganic nutrients and Chl *a* vary substantially amongst the different sub-basins and different activities of the plankton community (bloom phases).

Spilling et al. (2019) defined four bloom phases (Table 1 therein) for N- and P-limited sub-basins (Tamminen & Andersen 2007) that were used in the presented study.

Determination of the Chl *a* concentration

The protocol used was explicitly developed to determine Chl *a* in the Baltic Sea and is applied by the monitoring program organized by the Baltic Marine Environment Protection Commission ("Combine" marine monitoring manual, HELCOM 2017). The corresponding protocol was considered since data collected aboard R/V Aranda are saved in a database (Hertta, SYKE) and can be used for studies of long-term trends in the Baltic. Thus, the results collected in the Hertta database must be comparable amongst sampling programs, locations, and seasons. The same is valid for the plankton data.

Nano- and microplankton community composition and C-biomass

Besides planktonic organisms and their resting stages, all identifiable particles (including plant pollen) and organisms (for example benthic diatoms) that contributed to, for example, the pools of particulate organic carbon (POC) and Chl *a* were analyzed. The nano- and microplankton community composition was studied in all chapters, but different counting strategies were applied for field- and experiment samples. Field samples were analyzed according to the SYKE in-house protocol for saving the data in the Hertta database (details in Lipsewers & Spilling 2018) and to ensure the comparability of all plankton data for studies of long-term trends. For chapter 2, another counting strategy was followed, and a minimum of 400 cells was counted in each sample instead of considering a particular area of the counting chamber as done for field samples (details in Camarena-Gómez et al. 2018). The applied species identification guides are specific for the (phyto)plankton in the Baltic Sea and were used throughout the studies. Heterotrophic ciliates were not identified to high taxonomic levels. Merely, the small (~12.5-30 µm) and abundant *Lohmaniella oviformis* and tintinnids (higher biomass) were finally considered. Identification was done based on Gruner (1981) and https://denstoredanske.lex.dk/ciliater. For simplicity, the C-biomass of these and unidentified taxa were summarized to heterotrophic ciliates for further analyses. The abundances, biovolumes, and C-biomass were provided by a counting-software (EnvPhyto, SYKE) for field samples, and the same equations were considered for the experiment samples. For heterotrophic ciliates, these results were determined "by hand" based on counting protocols in Excel. Biovolumes were converted to C-biomass (microscopy derived carbon, MDC) using specific conversion factors for diatoms, dinoflagellates, and other protist plankton (Menden-Deuer & Lessard 2000), and heterotrophic ciliates (Putt & Stoecker 1989, Auf dem Venne 1994). Organisms >100 µm cell size were excluded from the analyses for chapters 1 and 2, except for some slightly larger cells of the diatom *T. baltica*. The C-biomass was used to calculate the diatom-dinoflagellate-proportion (DDP and Diat:Dino in chapters 1 and 2, respectively). Wasmund et al. (2017) developed the DDP as a sub-basin-specific index based on wet weight (mistaken with biovolume in chapter 2). In contrast, the MDC was used for the presented study and the DDP was calculated for each sample (DDP >0.5 = relative diatom-dominance, DDP <0.5 = relative dinoflagellate-dominance).

Gross primary production (GPP)

Spilling et al. (2019) recently published the details on incubations (2h, light), ^{14}C-measurements, dissolved inorganic carbon (DIC) analysis, and calculation of the GPP, specifically suggested for the Baltic Sea by Gargas (1975) and thus, these steps are not shown in Table 1. The only difference in the method used for both studies was the final

specific activity of the radiolabeled dissolved bicarbonate (0.2 µCi ^{14}C mL^{-1} for experiments, 0.1 for cruises).

Bacterial production

The bacterial incorporation of thymidine is a proxy for DNA (deoxyribonucleic acid)-synthesis, and leucine incorporation is a proxy for protein-synthesis. According to Camarena-Gómez et al. (2018), the final concentrations of thymidine and leucine, used to estimate the bacterial production (BPT and BPL, Table 1), are considered as saturating for the study area and the sampling season (winter / early spring). Samples spiked with radioisotopes were incubated for 2 hours in the dark. A blank to check for non-biological adsorption of the radioisotopes was analyzed.

3.3) Summary of statistical analyses

Detailed descriptions of the statistical analyses (considered data, data preparation and transformations, specific tests, R-packages / functions / plots, *etc*.) can be found in the different chapters. The various methods (including software versions and purpose) are summarized in Table 2.

Table 2 Summary of statistical analyses done for the presented studies. incl. = including, add. = additionally. All other abbreviations were explained previously. Note that details on additional tests within the main analyses, data preparation / transformation, data considered, complete list of software packages / functions, and plotting as well as the corresponding references, can be found in the separate chapters and are not repeated within the scope of this summary.

Ch.	Analysis	Software	Purpose
1	Linear and other regressions, curve fitting (r^2 and *p*-values), plots (bar diagrams, box-plots *etc*.)	Sigma Plot 10	Correlations of various biotic and abiotic variables
1	Analysis of variance (ANOVA) & post-hoc tests, data visualized as box-plots, considering, their median, confidence intervals, and outliers at different bloom phases	Sigma Plot 10 and 13 (SYSTAT)	Studying differences in nutrient / Chl *a* stoichiometry of the seston in different bloom phases
1	ANOVA-like permutation test for RDA model simplification (R-function "anova.cca"), part of the RDA below	RStudio 1.1.442 (RStudio Team 2015), R 3.4.4 (R Core Team 2014), for Windows	Step-wise comparison of the RDA models to find the most parsimonious one, considered in Ch. 1
1	Redundancy analyses (RDA), forward selection function	RStudio / R (see above)	Identifying environmental variables (abiotic & Chl *a*) significantly affecting the plankton community structure
1	Community ordination (nonmetric multidimensional scaling, NMDS)	RStudio / R	Studying links between plankton dominance patterns and POC:PON:POP:BSi: Chl *a* stoichiometry / GPP
1	Generalized additive models (GAM´s), as part of the NMDS above	RStudio / R	Explaining to which extent the seston nutrient / Chl a ratios and GPP are affected by the community composition
2	ANOVA with previous regression analysis (incl. Levene´s test and Tukey´s *b*-test)	IBM SPSS 23	Identifying effects of different treatments (= communities) on BPT, BPL, BA, DOC (2012),

			DON, and BCC (add. in 2013) during the phytoplankton- and the bacterioplankton bloom, respectively
2	NMDS-plot	RStudio / R	Visualizing the differences in bacterial community dynamics between the two experiments
2	Repeated measures permutational ANOVA (PERMANOVA)	PRIMER v. 6 (Clarke & Gorley 2006), using the add-on package by Anderson et al. (2008)	Testing if the BCC is significantly affected by the treatment (= community) [2013]
3	Linear regressions, curve fitting (r^2 and *p*-values), plots (bar diagrams, box-plot *etc*.)	Sigma Plot 13 (SYSTAT)	Quantifying the contribution of the unaccounted biomass in the Finnish phytoplankton monitoring program during the spring bloom; Correlations of POC and MDC (see below); Correlation of Chl *a* and the phytoplankton biomass
3	Statistic (Z), comparing the slopes of linear regressions	Sigma Plot 13 (SYSTAT), Statistic (Z) application / interpretation according to Paternoster et al. (1998)	Testing the correlation between POC and the MDC included in monitoring as well as the MDC of the total community, separately
3	Calculation of the maximum error for microscopy counts	Excel (Microsoft Office), Equation for maximum error in percent (%) according to Willén (1976)	Determining the error of the ciliate biomass estimates based on the maximum error of microscopic counts

Additional explanatory variables were analyzed (see three chapters) but not important for primary response variables such as the community composition and seston stoichiometry. Thus, they are not mentioned within the scope of this summary.

3.4) Dinoflagellate identification – Epifluorescence microscopy and quantitative (q)PCR

This study was not considered for the different chapters of the presented thesis. Still, due to its significance for phytoplankton research in the Baltic Sea, it was decided to include it in the presented summary. The relative abundances of dinoflagellates determined by both approaches were compared to see how well the methods correlate (linear regressions, SigmaPlot 10, Table 1).

Epifluorescence microscopy of stained cells

The microscopy approach to identify and quantify *G. corollarium*, *B. baltica*, and *A. malmogiense* (DinoComplex), is based on inverted epifluorescence microscopy of Calcofluor white MR2 (Fluorescence Brightener 28, Sigma-Aldrich) stained cells. The dye absorbs UV (ultraviolet)-radiation (340-400 nm range) and re-emits visible blue light (Fritz & Triemer 1985). Thus, the microscope equipment and settings must be selected accordingly. The amount of cellulose in the dinoflagellate cell wall determines the intensity of the stain. Therefore, differences between the three species can be visualized, and they can be distinguished by studying characteristic patterns and features of thecal plates. The staining

method is based on the different cell wall morphologies of the three suspects: *G. corollarium* is athecate and not stained at all; *B. baltica* has thin thecal platelets and appears uniformly blue under the microscope; *A. malmogiense* has thick thecal plates and features the most intense color. The original method was published by Fritz and Triemer (1985). The method used for the presented study was slightly modified and optimized for Baltic Sea samples by Anke Kremp. In this case, environmental samples preserved with neutral Lugol´s solution were considered, as Calcofluor is pH-dependent and does not work with acidic preservatives. The cells were concentrated, according to Utermöhl (1958). Based on the abundance of the DinoComplex (total counts in station samples), sedimentation volumes of 25 to 100 ml were used. Five drops (Pasteur pipette) of a Calcofluor white MR2 working solution (1 mg ml^{-1}) were added to the concentrated sample (Hydro-Bios counting chamber, 2.973 ml) after sedimentation (24-72 h). Samples were incubated for at least five minutes in the dark at room temperature before analysis with the aid of an inverted epifluorescence microscope (Leica DMI 3000 B) and a camera (Leica DFC 490) at 40x times magnification. It was aimed to count at least 100 cells of the dinoflagellate-complex per sample. The relative proportions of the three species were determined for samples from three cruises. By knowing the species-specific counts and the biomass of the complex (previously determined for each station), the relative contribution can be calculated as percent and as a biomass estimate. Monoalgal cultures of the three species (FINMARI culture collection) were used to test the fluorescent dye and to validate the identification of each species in field samples (strains: WHTV-S1 [*B. baltica*], GCTV-C1 [*G. corollarium*], SHTV-JR [*A. malmogiense*]).

Quantitative polymerase chain reaction (qPCR)

The qPCR protocol was developed by Brink et al. (2019, BioRxiv). A preprint version of the method is deposited here: https://biorxiv.org/cgi/content/short/871020v1. The ongoing optimization process and basic principles of this method are described in the supplementary materials of this summary. For this pilot study, positive and negative controls were added to the protocol to verify the specificity of the final reaction. For instance, DNA extracts of a *B. baltica* strain (BBTV-1401, FINMARI culture collection) were used as a positive control for *B. baltica*, and DNA of cultured *G. corollarium* (GCTV01) or *A. malmogiense* (SHTV-1) served as negative controls. The cultures were diluted (1:3) with filtered, artificial seawater, and 50 ml were filtered for DNA-extractions. The environmental drivers of the relative proportions of the different species, as well as their specific distribution patterns during the Baltic spring bloom, were investigated by multivariate analyses (see RDA in Table 2 and considered data in the results) and simple bar-plots considering the different sub-basins.

4.) Results

Previous studies have found that the phytoplankton community composition during the Baltic Sea spring bloom has shifted towards higher proportions of dinoflagellates relative to diatoms in several sub-basins, with implications for ecosystem functioning. Chapters 1 and 3 are based on data from four research cruises (4.1, 4.2, and 4.4), and chapter 2 is based on two experiments (4.3). In the following, only the main results are shown (summarized), and a complete description can be found in the different chapters. Additionally, the results of a pilot study (dinoflagellate identification) are shown.

4.1) Natural spring bloom in the Baltic Sea

Note that the samples from different bloom phases originate from different cruises (2013 to 2016). Considering the succession of the most relevant groups (diatoms, dinoflagellates, and

ciliates), it becomes evident that the total bloom-biomass of dinoflagellates and ciliates was higher relative to diatoms in the surface water (Fig. 3). The development of the nano- and microplankton community structure represented the natural succession and main features of the species composition during the Baltic Sea spring bloom (Fig. 3).

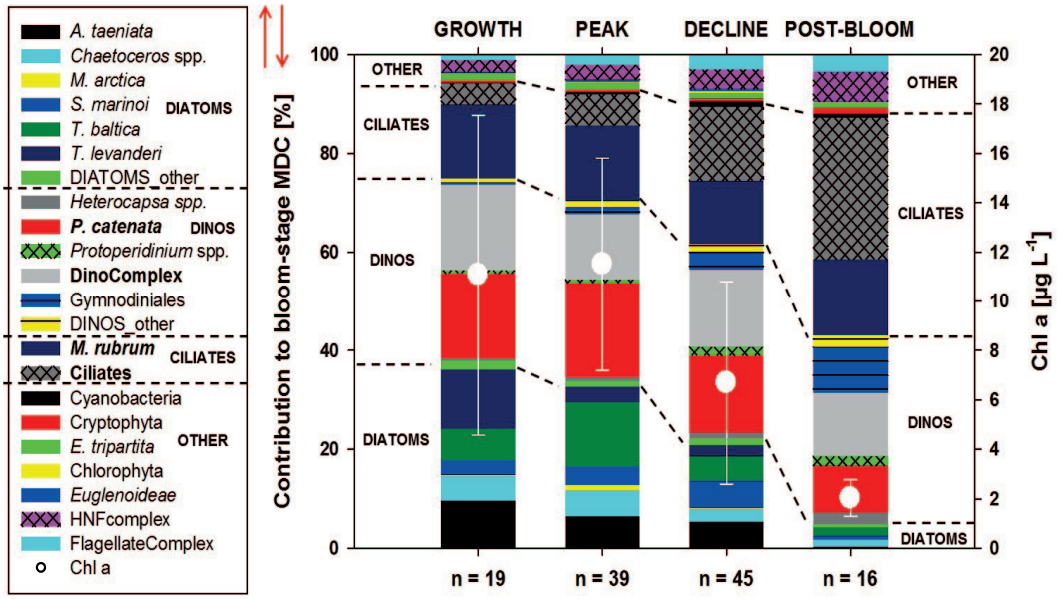


Fig. 3 Nano- and microplankton communities at different bloom phases. Stacked bars represent absolute contributions of the 22 species / groups to the microscopy derived carbon (MDC in %, left y-axis). The red arrows in the top-right corner of the legend, indicate that the order in the legend is opposite to the one in the stacked bars. The four most abundant species / groups, considering the whole dataset (n = 119), are bold-printed in the legend. Colors of stacks are repeating but not within one group (e.g., DIATOMS). Stacks with check pattern indicate heterotrophic organisms, line pattern indicates mixed groups, and the other ones represent phototrophic and mixotrophic organisms. Bloom phases are shown on the top x-axis and the number of samples (n) on the bottom x-axis. Dashed lines represent trends of the four main groups (DIATOMS, DINOS = dinoflagellates, CILIATES, OTHER) along the bloom. White circles represent the average Chl *a* concentration, and the error bars the standard deviation.

Considering the whole dataset (n = 119), five taxonomic units contributed >65 % (relative proportions) to the MDC: *Mesodinium rubrum*, *Peridiniella catenata*, heterotrophic ciliates, DinoComplex, and *Thalassiosira baltica* (in order of contribution from high to low). From the growth phase of the bloom to post-bloom conditions the phototrophic contribution (Chl *a*), dominated by diatoms, decreased drastically (indicated by dashed lines in Fig. 3). The proportion of heterotrophs (mainly ciliates and nanoflagellates [HNF]) increased. The diatom community changed the most: *Achnanthes taeniata* and *Thalassiosira levanderi* proportions were highest at the growth phase of the bloom; *T. baltica* and *Chaetoceros* spp. contributed their highest proportions during the peak phase; and *Skeletonema marinoi* was most abundant at the decline phase. During the growth phase, the diatoms contributed the same biomass-share as dinoflagellates (~40 %), whereas they were virtually absent (~5 %) at post-bloom phase conditions. The overall dinoflagellate-biomass (dominated by the phototrophs, *P. catenata* and DinoComplex) remained high (~40 %) and constant throughout the bloom, which was also valid for *M. rubrum* (~20 %). Heterotrophic dinoflagellates (mainly Gymnodiniales and *Protoperidinium* spp.) were more abundant in the post-bloom phase, but the phototrophic ones still dominated the community. The NMDS (see 4.2) supported these findings and has shown a link between the growing diatom population and the heterotrophic silicoflagellate *Ebria tripartita*.

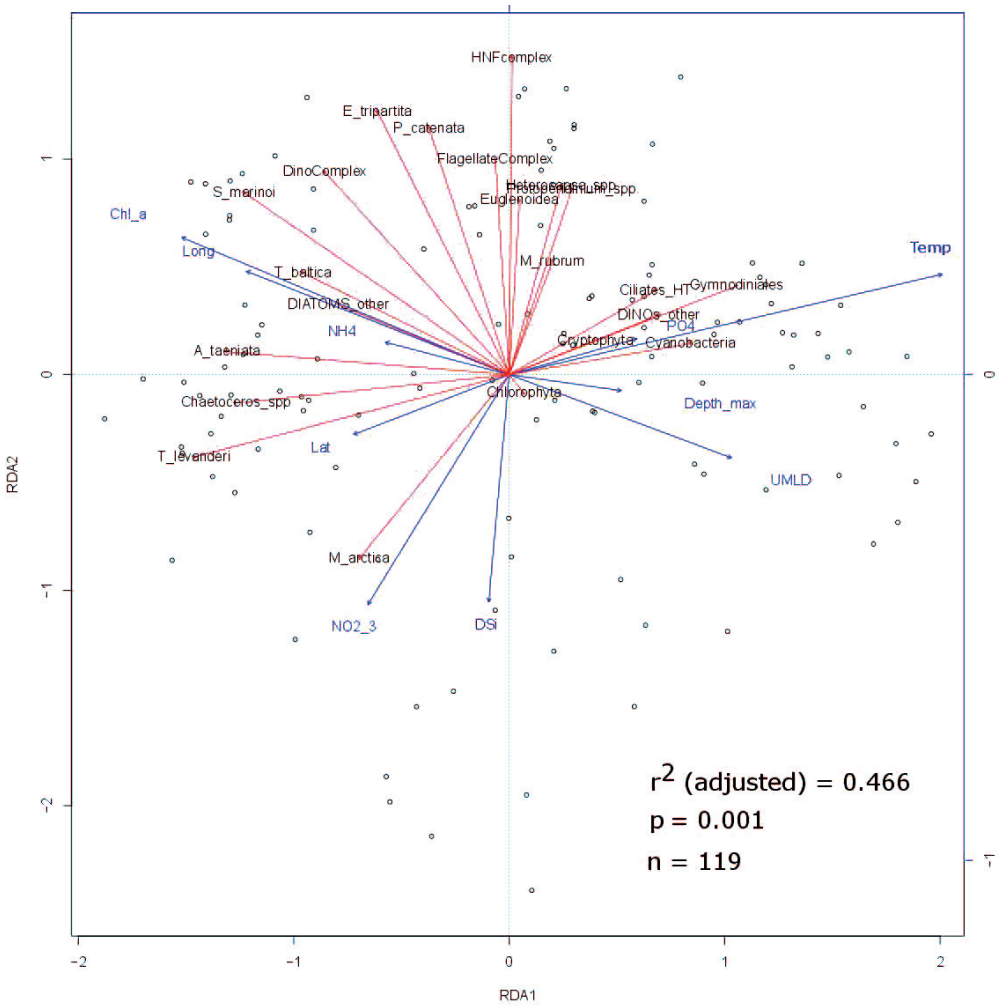


Fig. 4 Redundancy analysis (RDA) using forward selection with environmental variables significantly affecting the community composition (n = 119). The adjusted r^2 value (0.466, RDA) and the *p*-value (0.001, ANOVA-like permutation test) represent the most parsimonious model considered for this RDA-plot. Blue arrows (equivalent to r^2 values) represent explanatory variables: Temperature (Temp), silicate (DSi), latitude (Lat), Chlorophyll *a* (Chl_*a*), longitude (Long), depth of the upper mixed layer (UMLD), sum of nitrite and nitrate (NO2_3), bottom depth of the stations (depth_max, m), phosphate (PO4), and ammonium (NH4). Nutrients represent dissolved inorganic forms (µmol L^{-1}). Red arrows represent response variables: 22 nano-and microplankton organisms / groups based on their microscopy derived carbon (MDC, µg L^{-1}].

The RDA (Fig. 4) demonstrated that the described species succession was driven by environmental factors ($p \leq 0.006$), particularly temperature, UMLD, and the concentration of dissolved inorganic nutrients (mainly dissolved silicate [DSi]). A complete list of the environmental variables considered as well as their significance levels for the community composition, can be found in the supplementary materials of chapter 1. The highest plankton-biomass was linked to more coastal stations and the highly eutrophicated GOF (higher longitudes). Most diatoms were more abundant at lower temperatures, which was linked to the growth phase of the spring bloom and higher latitudes (BS). Especially early bloomers such as *T. levanderi* and *Melosira arctica* were more relevant at lower temperatures and higher concentrations of inorganic N and DSi, whereas late bloom representatives (heterotrophic ciliates, Gymnodiniales, cyanobacteria) correlated with higher temperatures and phosphate concentrations ($p = 0.022$, Fig. 4). The positive correlation of the UMLD and the bottom depth (depth_max, Fig. 4), suggests that thermal stratification was not well pronounced (deeper water = deeper mixing).

Excursion: Species-level identification of three cryptic cold-water dinoflagellates – The DinoComplex

This study focused on the identification and quantification of the three species included in the DinoComplex (*Gymnodinium corollarium*, *Biecheleria baltica*, and *Apocalathium malmogiense*), and the unpublished results are shown in the following.

The different appearances of Lugol-preserved cells (as seen usually) and when exciting the Calcofluor-dye with UV-light, allowing the separation of these species, are highlighted in Fig. 5. *A. malmogiense* shows the most intense signal (blue color) due to its thick thecal plates, the athecate *G. corollarium* is not stained at all, and *B. baltica* is uniformly stained due to its thin thecal platelets.

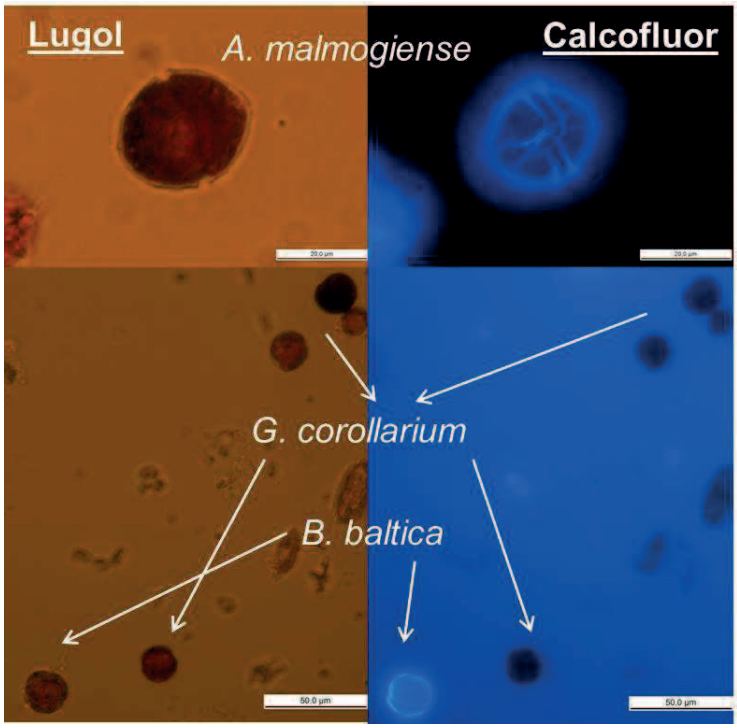


Fig. 5 Comparing the appearances of the three dinoflagellates in Lugol-preserved samples (left panel) to the Calcofluor-stain of the identical cells (right panel). Note the different measuring bars for *Apocalathium malmogiense* (20 µm) and the other species (50 µm). Images were taken at 40x magnification.

The comparison of the results obtained by microscopy and qPCR for *G. corollarium* and *B. baltica* resulted in significant positive correlations (linear regressions, n = 53, r^2 = 0.746 and 0.760, respectively; $p < 0.0001$, Fig. 6). Considering the samples taken in 2014 (n = 15), the correlation was even stronger (*G. corollarium* r^2 = 0.960, *B. baltica* r^2 = 0.972). The comparison of the results for *A. malmogiense* was not significant (n = 53, r^2 = 0.08, p = 0.538), since the microscopic method resulted in contributions of 0 % in most samples. In contrast, the qPCR is far more sensitive and detected this species more frequently. Nevertheless, the same trend (minor contribution) was shown by both methods.

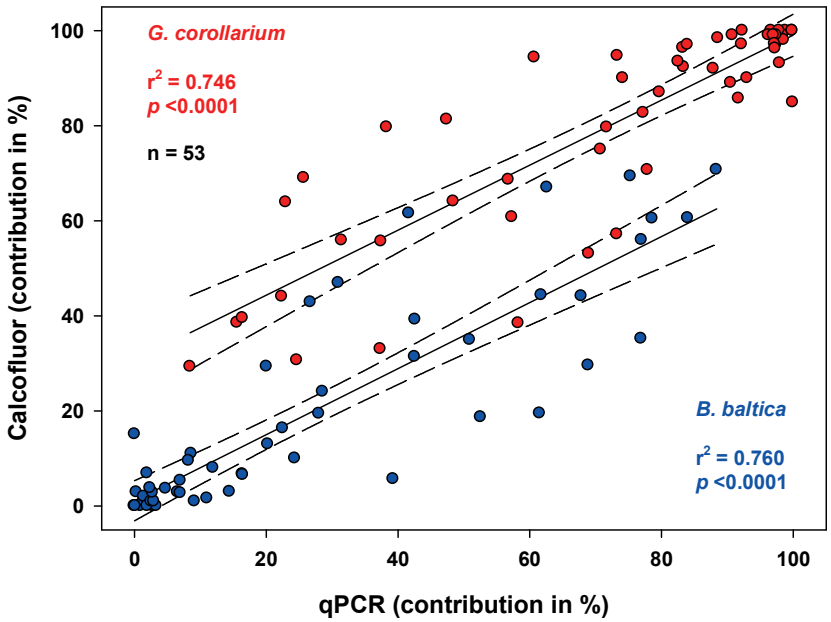


Fig. 6 Comparison of microscopy- and qPCR-results for *Gymnodinium corollarium* (red) and *Biecheleria baltica* (blue). Samples from the cruises in 2014 and 2015 were considered (n = 53). The solid lines represent the trendlines for the linear regressions, and the dashed lines indicate the 95 % confidence intervals. All correlations were based on the relative contributions (%) of the different species determined by both methods.

G. corollarium and *B. baltica* clearly dominated the DinoComplex. Considering sub-basins (Fig. 7) and bloom-phases revealed species-specific distribution patterns and different niches of these two species. Based on RDA, higher *G. corollarium* proportions correlated positively with higher salinities and lower latitudes, which were linked to the BP. Furthermore, this species was more abundant in the Archipelago Sea and later bloom phases. *B. baltica* correlated positively with higher Chl *a* levels and lower temperatures, which were linked to the GOF, the BS, and earlier bloom phases.

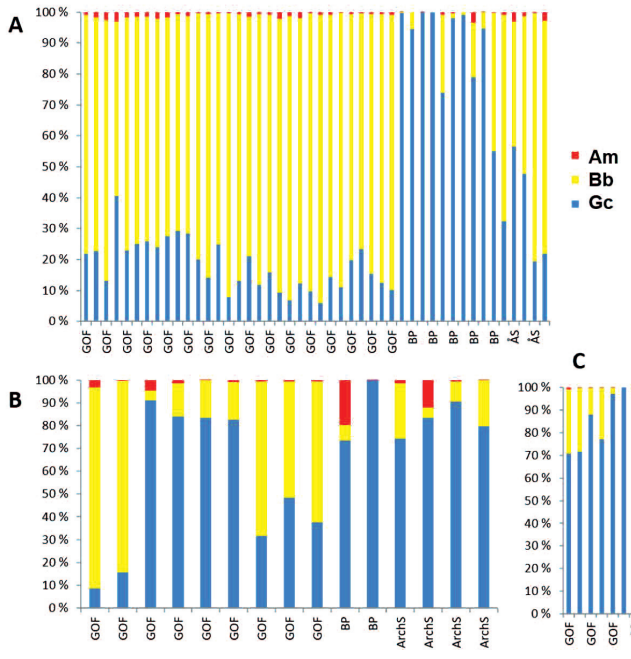


Fig. 7 The relative contributions (%) of three species summarized to the DinoComplex (Am = *Apocalathium malmogiense*, Bb = *Biecheleria baltica*, Gc = *Gymnodinium corollarium*) in different sub-basins of the Baltic Sea. The results are based on qPCR. A: 2013 (46 stations), B: 2014 (15 stations), C: 2015 (38 stations). See Fig. 2 for the abbreviations of sub-basins.

A. malmogiense (former *Scrippsiella hangoei*), contributed only a minor fraction of the dinoflagellate- biomass during the spring bloom. Except for two stations in 2014 (Fig. 7B), this species contributed clearly <10 % to the biomass of the DinoComplex.

4.2) Effects of natural communities on stoichiometric ratios of seston C:N:P:Si:Chl *a*

In the following, it will be referred to the stoichiometric seston ratios of C:N:P:Si:Chl *a*, based on the molar concentrations of particulate organic nutrients (e.g., POC:PON = seston C:N, *etc*.) and the concentrations in weight (µg L^{-1}) in case of Chl *a*:C.

The average seston C:N:P ratio (total 132:17:1) differed between the bloom phases: growth 103:14:1; peak 144:18:1; decline 136:17:1; post-bloom 127:17:1. The average ratios (± standard deviation, n = 119) were C:N 8.0±1.2, C:P 145.9±52.0, and N:P 18.0±5.1. Compared to peak and decline phases, the mean C:N and C:P ratios were lower and closer to the Redfield ratio at the growth phase. Generally, these ratios were most similar at growth and post-bloom phases (Fig. 8). The C:N and C:P ratios differed between the growth and peak phase of the bloom, and the latter also between the growth and decline phase (ANOVA, chapter 1). The mean N:P ratio was close to 16 (Redfield) at the post-bloom phase and slightly lower at the growth phase. This ratio differed between growth and peak and growth and decline. The seston C:Si and N:Si ratios were virtually constant along the bloom. The Chl *a*:C ratio and thus, the phototrophic contribution gradually decreased after the peak phase (Fig. 8A).

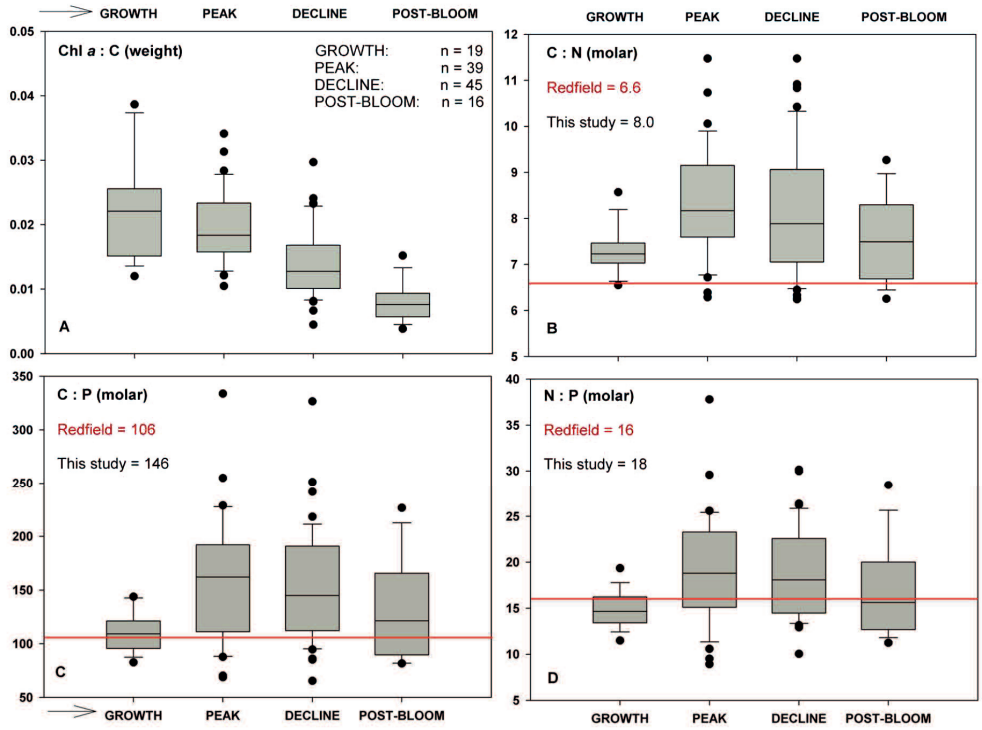


Fig. 8 Box-plots of four seston ratios (A: µg L^{-1}; B-D: mol L^{-1}) at different bloom phases (horizontal line in boxes = median, box = 25-75 percentiles, whiskers = 10-90 percentiles, dots = outliers). Plot A: Chl *a*:C, B: C:N, C: C:P, D: N:P. Note the individual y-axes do not start at zero. Numbers of samples from different bloom phases are given in plot A. Red lines indicate Redfield ratios (also stated in plots). Average ratios of this study (n = 119) are given as well. All ratios were significantly different between the bloom phases to different extents (ANOVA results in text). The average C:N:P ratio differed between the bloom phases: growth 103:14:1; peak 144:18:1; decline 136:17:1; post-bloom 127:17:1 (total C:N:P = 132:17:1, n = 119). The arrows indicate the course of the bloom from left to right.

The effect of the community composition on seston stoichiometry is exemplarily shown for the Chl *a*:C ratio (Fig. 9). The results for the other five ratios (see table in Fig. 9) are based on the same NMDS/GAM analyses (supplementary SFig. 3, chapter 1). The plankton community composition affected C:Si, N:Si, and Chl a:C (Fig. 9) most significantly and explained 48, 47, and 45 % of their variability, respectively. Generally, higher Chl *a*:C ratios (~0.02-0.03) were associated with higher contributions and diversity of diatoms and maxima (~0.04) were associated with the most relevant phototrophs (Dino-complex, *P. catenata*, *T. baltica*). The Chl *a*:C ratio had a fixed range (~0.005-0.04), irrespective of the taxon considered for the correlation. Despite their partially lower relative biomass-proportion, diatoms (26 % MDC) determined the Chl *a*:C ratio most clearly compared to dinoflagellates (30 % MDC) and M. rubrum (16 % MDC). The GPP was higher at diatom-dominated stations during the growth phase as well, and generally followed the same trend as Chl *a*:C.

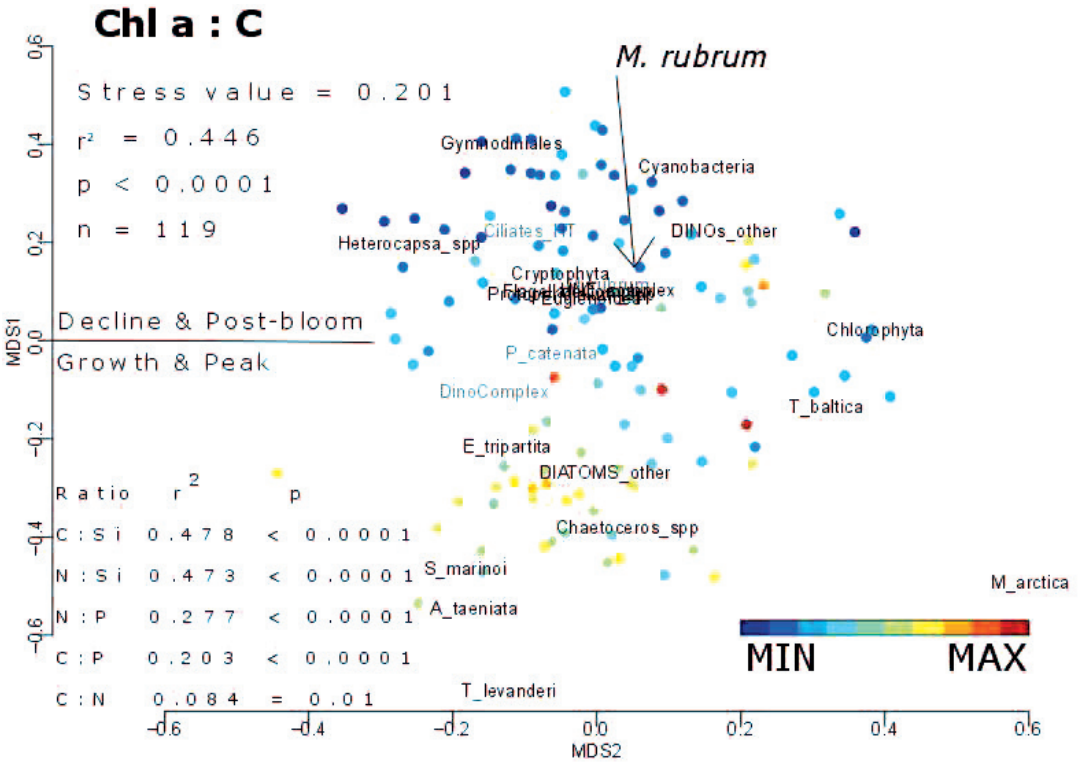


Fig. 9 Community ordination (NMDS) based on the C-biomass of the 22 plankton species / groups. The effect of the community composition on the Chl *a*:C ratio is shown as an example. The r^2 and *p* values shown in the figure were obtained by generalized additive models (GAM´s), using the coordinates of each community along the MDS1 as explanatory variables. Seston ratios were the response variables. The results for the other seston ratios are shown in the table in the lower left corner. Symbols are colored by the Chl *a*:C ratio (color scale: MIN = minimum, MAX = maximum). The black horizontal line separates bloom phases growth and peak from decline and post-bloom based on the community composition. The four most relevant species / groups are represented by grey labels (the arrow indicates the position of *Mesodinium rubrum*), and the others are labeled in black. Stress value (0.201), number of observations (n = 119), and the arrangement of species / groups were identical for all six seston ratios.

T. baltica significantly affected the C:Si and N:Si ratios, which exponentially decreased with increasing biomass of this diatom (chapter 1). The community structure explained 28 % of the variability in N:P, 20 % in C:P, and only 8 % in C:N ($p \leq 0.01$). The seston N:P ratio ranged from ~9 to 38 and increased at higher diversity and after the peak. The lowest values were associated with diatom-dominated communities (≤16), but slightly higher ratios were found at *T. baltica* dominated stations. Mostly C:P exceeded the Redfield ratio of 106 (65-334). Lower

values were found at higher biomass and diversity of diatoms (~65-132), and thus, during the growth phase. *T. baltica* dominated stations (peak phase) featured higher ratios (~200), and maxima (~200-330) were associated with higher contributions of certain dinoflagellates and the less abundant cyanobacteria (late bloom phases). At most stations, C:N was well above the Redfield ratio of 6.6 (mostly 6-12), but generally lower and closer to Redfield (6.2-7.6) at higher diversity and biomass of diatoms and again, higher ratios (8.9-11.5) were associated with *T. baltica*.

4.3) Dynamics of heterotrophic bacteria in different experimental phytoplankton communities

In the following, it will be referred to the different treatments (additions of cultured diatoms and dinoflagellates) as Diatom1 (*C. wighamii* / *T. baltica*, 2012), Diatom2 (*T. baltica*, 2013), Diatom3 (*A. taeniata*, 2013), Dino1 (DinoComplex / *P. catenata,* 2012), and Dino2 (*B. baltica*, 2013).

The initial (control) biomass was low (Chl *a* ≤0.2 µg L^{-1}, total MDC ≤2.7 mg L^{-1}), and all treatments featured <0.75 µg Chl *a* L^{-1} after adding the cultured strains in both years (Fig. 10). Heterotrophic ciliates were initially the most abundant members of the plankton community (2012: 40 % MDC, 2013: 80 % MDC). Unidentified flagellates and dinoflagellates (2012) and *P. catenata* (2013) contributed 15 to 20 %, whereas diatoms were least abundant (Fig. 10). These dominance patterns changed towards the Chl *a* peaks and, for example, ciliates were outcompeted by phototrophs in both years (<7.3 % MDC). In 2012, the peaks in control and Diatom1 were dominated by diatoms, and the biomass was lower in Dino1 (Fig. 10A). The latter was dominated (~90 %) by the DinoComplex, which was added to this treatment. In 2013 (Fig. 10B), the Chl *a* peak was comparable in both diatom treatments and the control, which featured very similar community compositions, whereas Chl *a* was higher in Dino2. The latter featured ~30 % of the added *B. baltica* and was dominated by diatoms. *T. levanderi* (not added) was present in low abundances in the natural water (both years) and contributed ~50 % of the MDC in almost all diatom treatments (except for Diatom1: >80 % added *Chaetoceros wighamii*) and in Dino2 (Fig. 10).

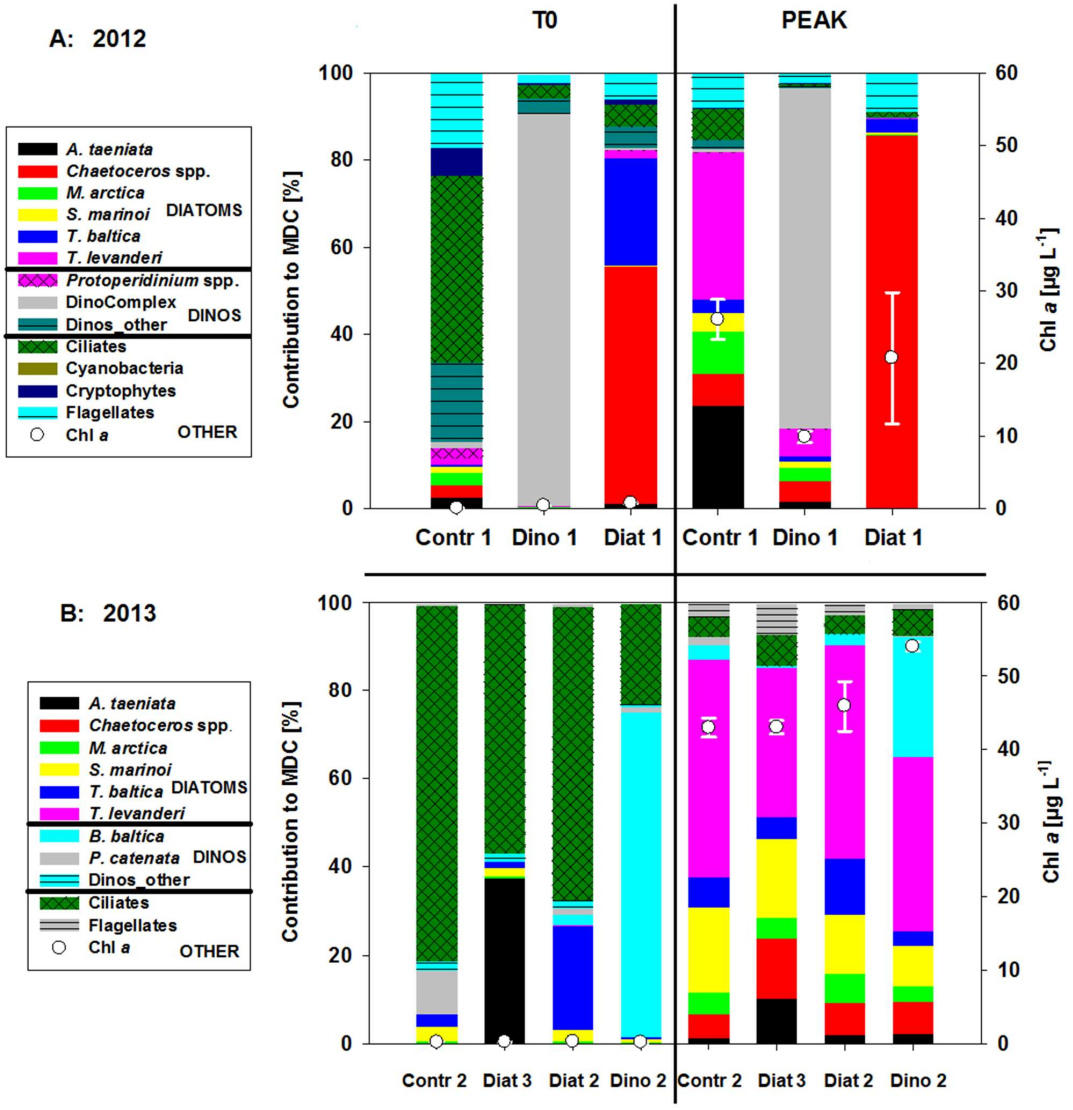


Fig. 10 The plankton community composition (relative contributions in %, considering species / groups >1 % MDC, stacked bars) in controls (2012: Contr 1, 2013: Contr 2) and treatments (Diat = diatoms, Dino = dinoflagellate) on day 0 (T0) [left panel] and the Chl *a* peak (Peak) [right panel] for the experiments in 2012 (A) and 2013 (B) (Camarena-Gómez et al. 2018, chapter 2). The results represent the mean of three replicates. The order from top to bottom is opposite in legends and plots. The check pattern indicates heterotrophs, line pattern mixed groups, and other stacks represent phototrophs. The average (n = 3) Chl *a* concentration (white circles) and its standard deviation (error bars) is shown for all treatments. In 2012, cultured cells of *Chaetoceros wighamii* and *Thalassiosira baltica* (Diat 1) and *Gymnodinium corollarium*, *Biecheleria baltica*, *Apocalathium malmogiense*, and *Peridiniella catenata* (Dino 1) were added to shift the natural community. In 2013, only one species was added per treatment (Diat 3: *Achnanthes taeniata*, Diat 2: *T. baltica*, Dino 2: *B. baltica*). *B. baltica* represented the DinoComplex rather than only one species in controls (2012 and 2013) and Diat 2 and 3 in 2013.

All organisms considered for Fig. 10, contributed >1 % of the total MDC. Most of the cultured strains (*P. catenata*, *T. baltica*, and *A. taeniata*) contributed <5 % at the corresponding Chl *a* peaks.

The bacterial DNA-synthesis (thymidine-incorporation, BPT) was significantly higher in Diatom2 compared to the control and Dino1 during the second part of the experiment (bacterial growth phase) in 2012 (Fig. 11A). The same was valid for the bacterial protein-synthesis (leucine-incorporation, BPL), which was significantly lowest in Dino1 also (Fig. 11C). In 2013, a clear increase in bacterial DNA-synthesis in Dino2 (dominated by diatoms) during the bacterial growth phase, and a significant increase during both bloom phases in Diatom3 were detected (Fig. 11B). In this experiment, the thymidine/leucine-incorporation peak (BPT and BPL) in the diatom treatments was ~2-fold lower compared to 2012. In 2013, no significant increase in bacterial protein synthesis was observed in Dino2 throughout the whole experiment, whereas it increased during the bacterial growth phase in both diatom treatments, and also before in Diatom3 (Fig. 11D).

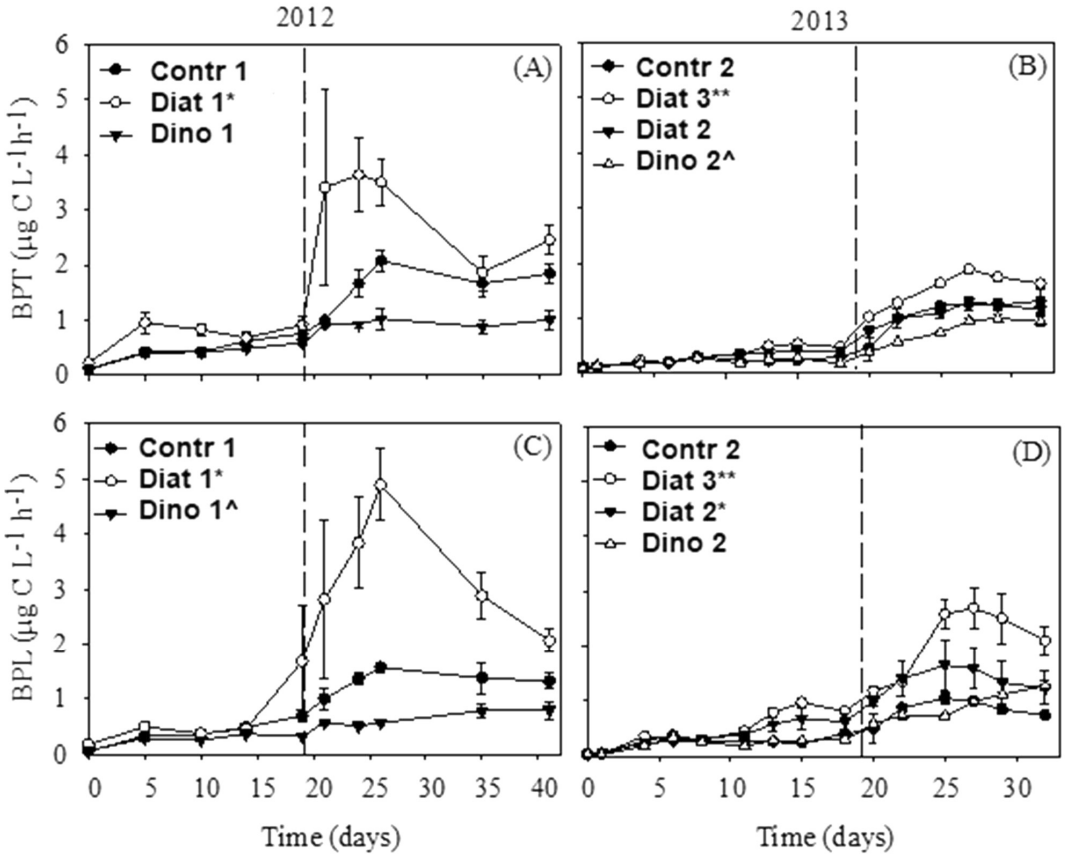


Fig. 11 Simultaneous incorporation of thymidine (BPT, figures A and B) and leucine (BPL, figures C and D), during the experiments in 2012 (left panel) and 2013 (right panel) (Camarena-Gómez et al. 2018, chapter 2). Contr1/2 = controls in 2012/2013, Dinoflagellate (Dino) 1 = DinoComplex and *Peridiniella catenata*, Diatom (Diat)1 = *Chaetoceros wighamii* and *Thalassiosira baltica*), Diat 2 = *T. baltica*, Diat 3 = *Achnanthes taeniata*, Dino 2 = *Biecheleria baltica*. The dashed lines indicate the increase in temperature from 4 (phytoplankton bloom) to 10 ^{0}C, to increase bacterial growth on day 19. The symbols * and ^ indicate significantly different substrate-incorporations between the treatments during the bacterial growth phase. Treatment Diat 3, indicated with ** featured significantly different incorporations between the treatments during both bloom phases. Note the differences between BPT and BPL. The significance levels ($p < 0.05$) were determined with Tukey´s *b*-test. The results represent the mean (n = 3), and the error bars the standard error.

The BCC shifted along the course of both experiments (Fig. 12A and B), and Alphaproteobacteria dominated all treatments at the beginning of the experiments (40-50 % relative abundances). In 2012 (Fig. 12A), also Beta- and Gammaproteobacteria were relevant on day 0 (~10 % each). The Betaproteobacteria increased from day 0 towards the Chl *a* peak

(day 19, Fig. 12A) in the control (Contr 1) and the dinoflagellate treatment (Dino 1) and remained rather constant in the diatom treatment (Diat 1). The Gammaproteobacteria increased from day 0 to day 19 in the control and Diatom1, whereas they remained constant in Dino1. Flavobacteria (maximum 60 %, Diatom1) and Alphaproteobacteria (maximum 30 %, Dino1) dominated the bacterial community on day 19 in all treatments. The latter remained fairly constant until the end of the experiment (day 41), the Flavobacteria decreased, Actinobacteria became more abundant (maximum 25 %, control), and four classes (Alphaproteobacteria, Betaproteobacteria, Actinobacteria, and Flavobacteria) dominated (80 %) in Diatom1. Additionally, cyanobacteria featured their maxima (considering both experiments) in Dino1 and the control (maximum 20 %) at the end of the experiment (day 41, Fig. 12A). In 2012, samples from replicate treatments were pooled, and the BCC was not statistically analyzed.

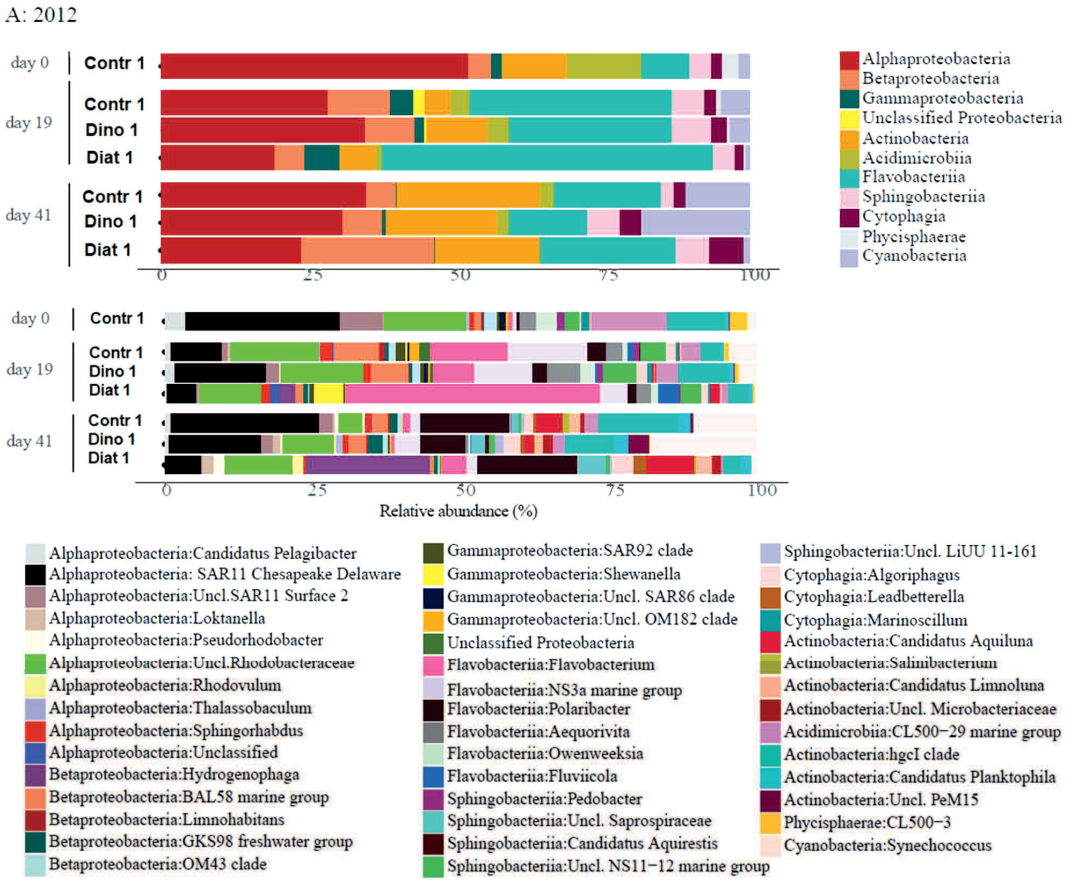
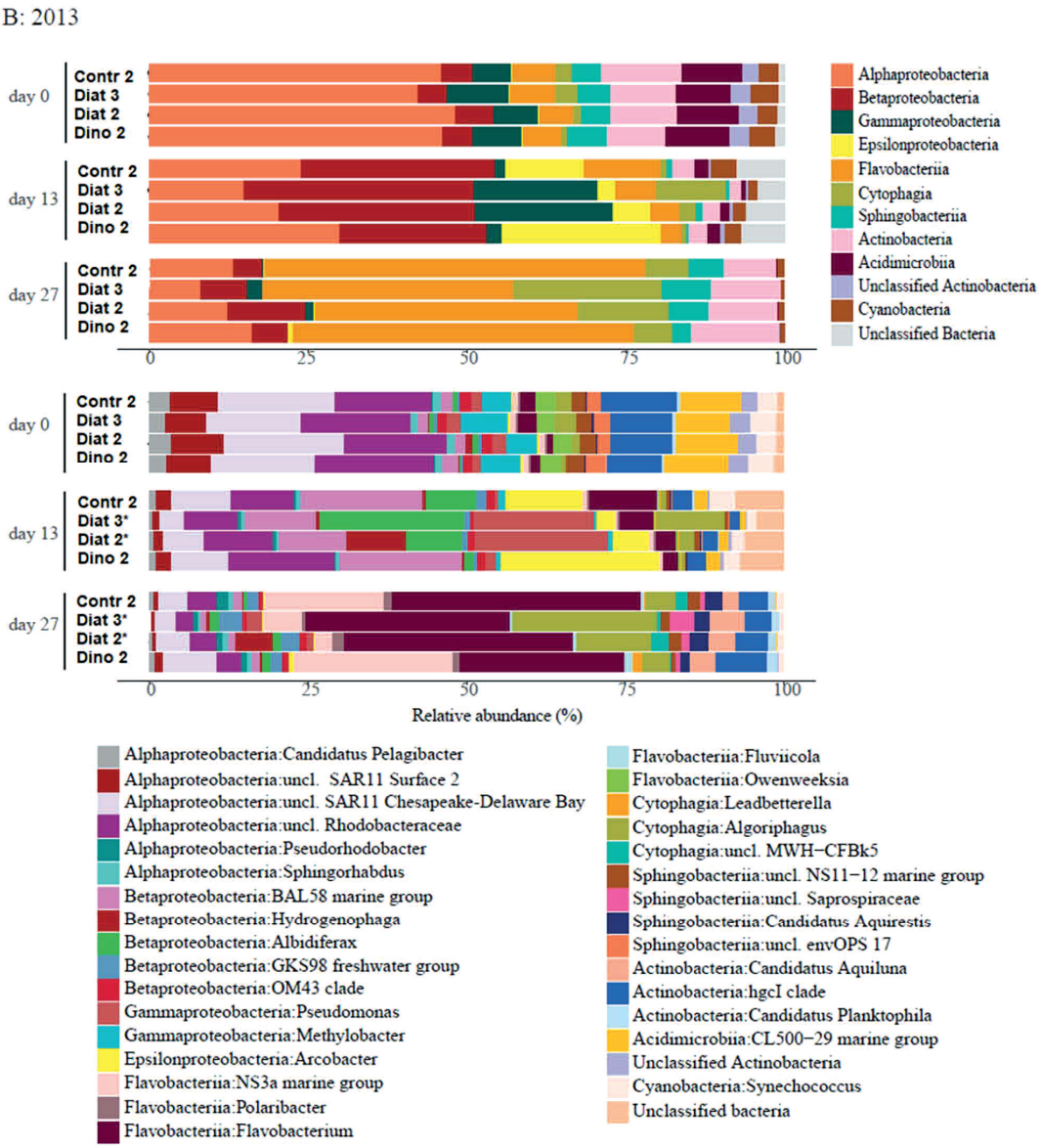


Fig. 12 (part B on next page) The bacterial community composition (BCC, contributions in relative abundances) at class- (upper figures in A and B) and genus-level (lower figures) in 2012 (A) and 2013 (B) based on differences in the 16S ribosomal(r)RNA sequences (Camarena-Gómez et al. 2018, chapter 2). B: The * indicates treatments (Diat 1 and 2) with significantly different BCC´s compared to control and Dino2 over time (n = 3, $p < 0.05$, PERMANOVA). The treatment labels were explained before (Fig. 10/11). The BCC in controls and treatments is shown for different time points: start of experiments (day 0), Chl *a* peak (A: day 19, B: day 13), and a later time point (A: day 41 = end of experiment, B: day 27 = bacterial production peak). In 2012, there were no replicates of the treatments, and thus, no statistical tests were applied.



Besides the predominant Alphaproteobacteria, Actinobacteria and Acidimicrobiia were relevant classes (≤15 %) on day 0 in 2013 (Fig. 12B). At the Chl *a* peak (day 13), these classes had decreased and Beta- (generally more relevant, maximum 35 % in Diatom3), Gamma- (more abundant in diatom treatments, 20 %), and Epsilonproteobacteria (especially more important in Contr2 and Dino2, 20 %) were more abundant. Furthermore, Cytophagia featured a relevant share (10 %) in Diatom3 and only three classes (Alpha-, Beta-, and Epsilonproteobacteria) dominated in Dino2 (80 %, Fig. 12B). By the time of the thymidine/leucine-incorporation peak (day 27), the previous main classes, and Alphaproteobacteria had declined, Flavobacteria dominated all treatments (maximum 60 %, control), with relevant contributions of Cytophagia (≤25 %) in both diatom treatments and Actinobacteria in all treatments (10-15 %). For this experiment, BCC samples were taken from all individual mesocosms, and statistical tests were applied. Based on PERMANOVA, the BCC (genus-level) was significantly different between the control and both diatom treatments (Diatom2: $p = 0.021$, Diatom3: $p = 0.027$) throughout the experiment (considering all three sampling days, Fig. 12B). The significance levels were higher when comparing the

diatom treatments with Dino2 (Diatom2: p = 0.013, Diatom3: p = 0.006). These findings are supported by the fact that the phytoplankton community composition was similar in diatom treatments and control and different in Dino2 (Fig. 10B).

During both experiments, the abundances of heterotrophic bacteria increased, especially after increasing the temperature (Fig. 6 in chapter 2). In general, higher abundances were found in diatom-dominated communities. In 2012, the bacterial abundances in Dino1, which was absolutely dominated by DinoComplex, increased only slightly, whereas the increase was far more pronounced in Diatom1 and the control. In 2013, the bacterial cell numbers followed the same trend as in Dino1 (slight increase) and were very similar in all treatments. Only on the last day, the abundances were clearly higher and very similar in the control and Diatom3, and ~50 % lower in Diatom2 and Dino2. Thus, the bacterial abundances generally followed the same trend as the primary production and Chl *a* (details in chapter 2). See https://www.int-res.com/articles/suppl/a081p149_supp.pdf (supplementary materials of chapter 2) for additional information.

4.4) Composition and biomass of microzooplankton during the Baltic Sea spring bloom

In the field data, heterotrophic ciliates dominated the microzooplankton community, and contributed the largest C-biomass at post-bloom conditions (e.g., higher temperature, Fig. 3). The contribution of unaccounted biomass was compared with the biomass obtained when following the counting protocol used by the Finnish phytoplankton monitoring program. The latter considers largely phytoplankton, but also some heterotrophic flagellates. Heterotrophic ciliates (<100 µm) contributed ~70 % of the MDC, and thus, clearly dominated the unaccounted biomass (Fig. 13A). The group “Other” (Fig. 13A) consisted of, for example, benthic diatoms and unidentified plankton resting stages. At most sampling stations, ciliates <100 µm contributed the highest heterotrophic biomass considered for this study, whereas cells >100 µm were found only occasionally (Fig. 13B). The group “Other plankton” (Fig. 13B) includes the species recorded by the Finnish monitoring program plus the unaccounted biomass (except for heterotrophic ciliates). Cells >100 µm were considered for this study to have at least an estimate on their contribution. However, because of the significant error of the biomass estimate for large and rare taxa detected with the chosen method, these cells were excluded for the other studies (details in chapter 3). The average maximum error was ±63.2 % (n = 125) and reflected the high variation in counted cells per sample. The ciliates >100 µm are in principle included in the mesozooplankton monitoring that samples with a 100 µm mesh net, but the community <100 µm is not recorded completely in regular Baltic Sea monitoring programs.

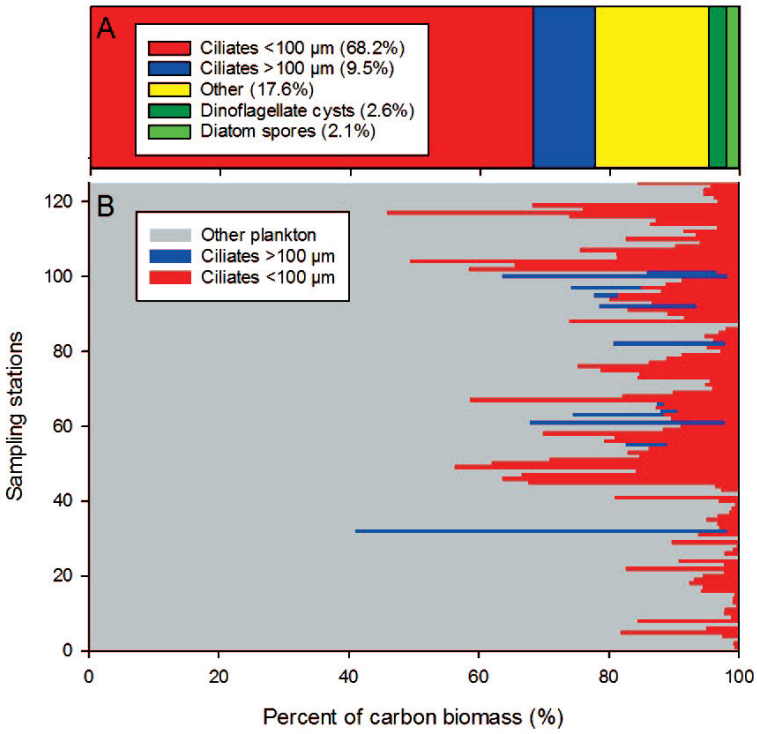


Fig. 13 A: The average (n = 125) proportion of different groups of unaccounted particles that are not included in the counting protocol of the Finnish phytoplankton monitoring program. The group "Other" included, for example, benthic diatoms and unidentified plankton resting stages. B: The contribution of heterotrophic ciliates to the total plankton biomass (phytoplankton monitoring plus extra counts) at all sampling stations. The group "Other plankton" included all organisms considered by the monitoring program and, the unaccounted biomass, except for heterotrophic ciliates.

On average, the MDC obtained by the Finnish monitoring program increased by 22 % (n = 125), when considering all identifiable organisms / particles shown in Fig. 13A. The biomass of ciliates made up the major part (mean±maximum error = 14.1±3.7 %) of this unaccounted fraction. At 10 % of the stations the total unaccounted biomass was ≥60 % and occasionally heterotrophic ciliates contributed >40 % (<100 µm, Fig. 13B). There was no apparent difference in this biomass between the open sea and coastal (<1 nautical mile from the mainland) areas.

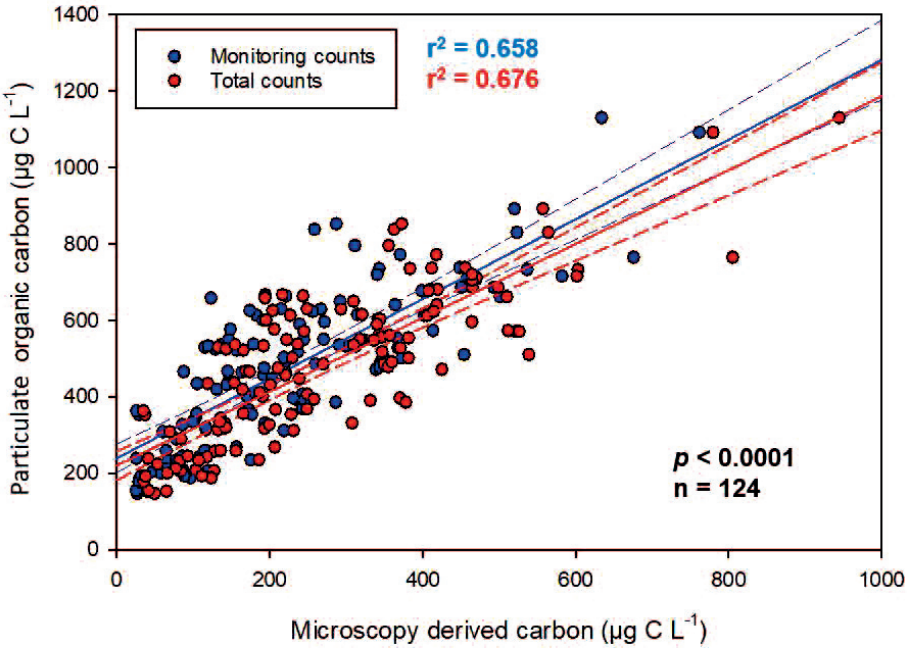


Fig. 14 The phytoplankton monitoring counts (blue), and total microscopy derived carbon (MDC, red) plotted against the particulate organic carbon (POC, n = 124). The solid lines represent the trendlines of the linear regressions and dashed lines the 95 % confidence intervals. Monitoring counts: slope = 1.04, r^2 = 0.658; total MDC: slope = 0.97, r^2 = 0.676 (both p < 0.0001).

The POC correlated significantly (n = 124) with the MDC obtained with the Finnish monitoring protocol and with the total MDC (including unaccounted biomass). Both correlations (Fig. 14) featured an almost identical positive slope (1.04 and 0.97, respectively). The monitoring MDC and Chl *a* correlated positively as well (n = 127). As the phototrophic contribution to the unaccounted biomass was insignificant, only the monitoring MDC was compared to Chl *a* (details in chapter 3).

5.) Discussion

The limitations due to the experimental set-up (chapter 2) and the fact that the field samples (chapters 1 and 3) represent an extract of natural processes were explained in the separate chapters. In the following, all main findings from field studies and experiments will be linked and their implications for ecosystem functioning and environmental changes in the future, discussed. Due to the common features of these different approaches, the results complemented each other and were combined in a conceptual model (Fig. 15).

5.1) Interactions between abiotic factors, phototrophic plankton, and heterotrophic plankton

Different members of the plankton community prefer different environmental conditions. Compared to dinoflagellates, diatoms feature higher growth rates at low temperatures, high inorganic nutrient concentrations, and turbulent (mixed) conditions (Margalef 1978, Smayda & Reynolds 2001, Spilling & Markager 2008, Finkel et al. 2010). These conditions are characteristic for the early phase of the spring bloom in the Baltic Sea and determined the community composition in the presented study. Higher biomass was linked to shallower mixed

layers in coastal waters, which are generally more productive than open waters (Brink 2004). Furthermore, diatoms sediment rapidly after inorganic N is depleted (von Bodungen et al. 1981, Heiskanen 1998), which is the dominant loss process for phytoplankton during the Baltic Sea spring bloom (Reynolds & Wiseman 1982, Riebesell 1989). These findings are supported by the results shown in chapter 3. The concentration of detritus in the seston was constant throughout the bloom, and its proportion of the total organic C-pool is expected to decrease with increasing biomass in the surface water, related to its rapid aggregation and sedimentation. In the proposed conceptual model (Fig. 15), this is indicated by the drop in the phototrophic proportion (mainly diatoms) and total biomass (Chl *a* and POC) after the bloom peak (Fig. 15).

Until then, diatoms and dinoflagellates dominated the nano- and microplankton community (equal proportions) in the field samples. In contrast, the dominance patterns were almost absolute in the experiments (≥80 % of either diatoms or dinoflagellates). In the cruise data, *Thalassiosira baltica* was the most relevant diatom, whereas *Thalassiosira levanderi* became dominant in most of the treatments during the experiments, despite its minor biomass proportion on day 0. *T. levanderi* is a small centric diatom and considered as an early-bloomer, growing fast at high nutrient concentrations (e.g., Höglander et al. 2004). The experiments were started with the natural winter-time high nutrient concentrations and in combination with the improved (artificial) light conditions, allowed this rapid growth. Interestingly, *T. baltica* dominated stations (peak phase) featured the lowest primary production detected during the cruises (presented study) and also low bacterial activity and diversity, which were comparable to dinoflagellate dominated communities (Camarena-Gómez et al. 2019, bioRxiv). These samples originated from the peak phase, only after which the concentration of labile DOM in the pelagic starts to increase and boost bacterial activity. The presented findings indicate that certain organisms, especially the ones contributing relevant proportions to the vernal biomass, require more attention in the future to study their effects on ecosystem functioning.

Overall, there was a development from a phototrophic (mainly diatoms and dinoflagellates), towards a more heterotrophic community (high proportions of heterotrophic ciliates) along the natural bloom. Generally, higher proportions of smaller taxa at later bloom phases are due to their larger surface-to-volume ratio (e.g., Lindemann et al. 2016), allowing more efficient nutrient uptake. Higher numbers of grazers usually accompany increasing abundances of prey organisms, such as bacteria (Alonso-Sáez et al. 2007, Camarena-Gómez et al. 2018). The heterotrophic silicoflagellate *Ebria tripartita* feeds on diatoms (Hargraves et al. 2002) and contributed only <2 % to the MDC. However, at higher biomass this species could be an important link to higher trophic levels, when mesozooplankton abundances are still low. Thus, *E. tripartita* could play a similar role than heterotrophic ciliates at later bloom phases (Mironova et al. 2012, Lipsewers & Spilling 2018). According to previous studies (Lignell et al. 1992, Mironova et al. 2012), grazing by heterotrophic ciliates can significantly reduce the abundances of phytoplankton and bacteria in the warmer season. High contributions of microzooplankton (heterotrophic ciliates, 20 % MDC) were found already during the decline phase of the bloom (chapters 1 and 3), and this group could become more abundant in the future. As heterotrophic ciliates are an essential part of the pelagic food web (Calbet 2008), it is recommended to start a microzooplankton monitoring program for the Baltic Sea. Currently, heterotrophic ciliates (including tintinnids) are neglected (Wasmund 2020, personal communication), and especially these organisms dominate the microzooplankton in spring (chapter 3). However, a monitoring program requires the development of a specific method to cover this group reliably. For instance, heterotrophic ciliates could be separated from smaller cells and detritus by sieving. Furthermore, the enrichment of desired organisms and size-fractionation could be used to enable microzooplankton counting in phytoplankton monitoring samples (Wasmund 2020, personal communication).

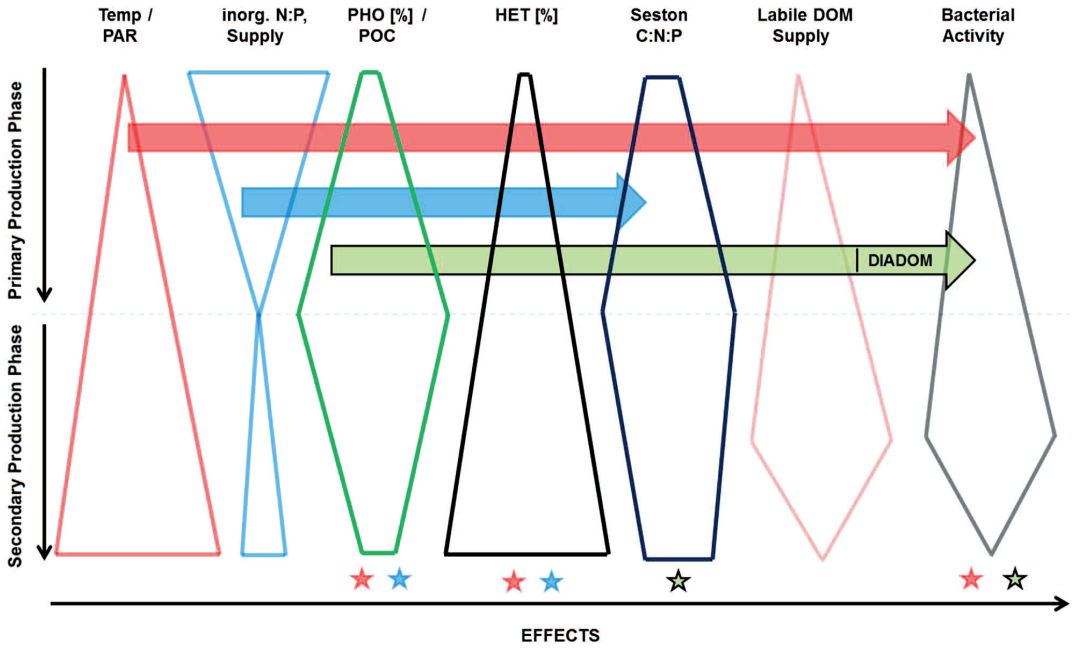


Fig. 15 Conceptual model of the interactions (effects) between the abiotic and the biotic world in the pelagic during the Baltic Sea spring bloom derived from the combined findings of the presented field studies and experiments. The stars under the figures indicate the interactions (matching color with arrows), studied for this thesis. The colored arrows (red = Temp, blue = inorganic N:P, green with black line = phototrophic and heterotrophic organisms) indicate the direction of the effects. The arrows pass the variables affected (e.g., Temp affects all the variables shown). The black line around the green arrow indicates that both phototrophic and heterotrophic organisms affect seston C:N:P, labile DOM supply, and bacterial activity. The fact that DIADOM affects the bacterial activity to a large extent, is indicated by its position at the end of the arrow. The dynamics of the variables along the bloom and with increasing temperature are indicated by the change in figure shapes along the y-axis (top: primary production phase, bottom = secondary production phase). The dashed line indicates the peak of the bloom and separates the two production phases. The labile DOM supply and PAR were not measured for the presented study. Temp = temperature, PAR = photosynthetically active radiation, inorg. = inorganic, PHO = phototrophic, POC = particulate organic carbon, HET = heterotrophic, DOM = dissolved organic matter, DIADOM = diatom-derived dissolved organic matter (DOM).

The experimental findings indicated that bacteria can thrive and proliferate at depleted inorganic nutrient concentrations, given that the temperature is suitable, and that the labile DOM supply is sufficient, and thus, no arrow was drawn from the inorganic nutrients to bacterial activity. Fig. 15 is based on the findings from the presented field studies and experiments, representing the processes in surface waters during the Baltic spring bloom with steadily increasing temperatures. All field samples were taken from the upper mixed layer, and the water in the mesocosms was mixed as well. The upper half of the proposed conceptual model (Fig. 15) represents the primary production phase, which is equivalent to the phytoplankton growth phase (chapter 2), including growth and peak phases (chapter 1), dominated by phototrophic processes, and characterized by decreasing inorganic nutrients as well as increasing biomass. The lower half comprises the secondary production phase and represents the bacterial growth phase (chapter 2) and includes decline and post-bloom phases (chapter 1), dominated by heterotrophic processes. The increasing temperature (red arrow, Fig. 15) and increasing photosynthetically active radiation (PAR, not detected), in combination with high concentrations of inorganic nutrients causes the onset of the spring bloom in the Baltic Sea (chapters 1 and 2) (e.g., Neumann et al. 2012). The arrow from the figure representing the phototrophic contribution to the bacterial activity (Fig. 15) has two colors: the green fill and the black line indicate that the whole plankton community affects the seston C:N:P ratio, labile DOM supply, and bacterial activity. However, the fact that diatom-derived dissolved organic matter (DIADOM) largely determines the bacterial activity is reflected by the position of the label in the corresponding arrow. The Chl *a*:C ratio followed

the same trend as the phototrophic contribution (Fig. 15), as it is largely determined by diatoms (details below). The blue arrow represents the inorganic N:P ratio and overall nutrient supply. The flat bottom end of the N:P figure (Fig. 15) indicates the surplus phosphate (PO_4^{3-}) available after the spring bloom in several Baltic Sea sub-basins (especially GOF) (Tamminen & Andersen 2007). The course of the relative phototrophic contribution also represents the total biomass (POC and Chl *a*, including *M. rubrum*) and GPP, both higher in growing diatom populations and decreasing after the peak (chapters 1 and 2, Spilling et al. 2014). The stars in Fig. 15 indicate that the effects of abiotic factors on the whole plankton community, the effect of the community composition on seston C:N:P, as well as the impacts of environmental factors (temperature and labile DOM source) on bacterial activity, were investigated.

Labile (readily bioavailable) DOM was not measured, but due to the high diatom-proportion (growth and peak phase, field data) and clear differences in bacterial activity between diatom- and dinoflagellate-dominated communities (experiments), the development shown in Fig. 15, and that the OM-pool was dominated by DIADOM (e.g., highly labile polysaccharides, Myklestad 2000), was assumed. Bioavailable DOM is rapidly taken up by heterotrophic bacteria (Azam et al. 1983, Smith et al. 1995), which stimulates their activity and proliferation (Riemann et al. 2000, Fandino et al. 2001, Buchan et al. 2014). The bacterial activity, and thus, also the energy efficiency of the microbial loop increased with increasing labile DOM levels in the experiments (chapter 2). The turnover time of the most labile compounds in marine ecosystems is only several hours (Keil & Kirchman 1999, Skoog et al. 1999). Thus, high-frequency sampling is required to detect the labile fraction of the DOM-pool against the high background, which is supported by the development of the total DOC along the experiments (details in chapter 2). The DOC-concentration increased after the phytoplankton bloom, followed by a slight decrease, probably because the labile fraction was taken up quickly, and thus, contributes a smaller fraction of the total DOC measured. The trends and concentrations of total DOC were not affected by the treatments, suggesting that the quality (composition) of the bioavailable DOM was responsible for the different bacterial responses to different phytoplankton dominance patterns.

In fact, the amount and composition of the labile DOM pool affected the community composition, abundances, and activity of heterotrophic bacteria (chapter 2). Along the phytoplankton bloom in the first experiment, the initial bacterial community composition (mainly Alphaproteobacteria) changed to high proportions of Flavobacteria. In 2013, Beta- and Gammaproteobacteria dominated the diatom treatments (peak), whereas Beta- and Epsilonproteobacteria were the most relevant classes in the other communities. Flavo- and Gammaproteobacteria are already found during bloom events, and at post-bloom conditions (Teeling et al. 2012, Laas et al. 2015, Bunse et al. 2016). These classes do not compete for the same resources (Teeling et al. 2012). Flavobacteria can use complex high molecular weight compounds (Cottrell & Kirchman 2000, Kirchman 2002, Buchan et al. 2014), whereas Gammaproteobacteria thrive at high concentrations of low molecular weight compounds (Eilers et al. 2000, Pinhassi & Berman 2003, Gómez-Consarnau et al. 2012). Betaproteobacteria use similar DOM sources compared to Gammaproteobacteria (e.g., Cottrell & Kirchman 2000) and inhabit estuaries with high DOM levels (Bouvier & del Giorgio 2002). Furthermore, phytoplankton species-specific effects on the bacterial activity were observed. The genus *Chaetoceros* is known to release more polysaccharides than other diatoms (Myklestad 1995), but it (mainly the added *Chaetoceros wighamii*) also absolutely dominated the community (~90 %) at the Chl *a* peak (chapter 2). Additionally, differences between *T. levanderi* dominated and more diverse diatom communities were found. Thus, changing phytoplankton communities affect the BCC through the quality and quantity of the DOM they produce (Pinhassi et al. 2004, Landa et al. 2014, Sarmento et al. 2016).

Minding these complex interactions leads to a basic question: What do the changes to the BCC mean for ecosystem functioning? More important than the apparent effects of changing

DOM pools on the bacterial community structure is what it means for the microbial loop, and thus, the collective activity of the community. The bacterial protein- and DNA-synthesis (activity and growth), was higher in diatom-dominated communities (chapter 2). If the share of DIADOM decreases, for instance, by decreasing DDP´s (Wasmund & Uhlig 2003, Klais et al. 2011, Klais et al. 2013), also the material fluxes within the microbial loop will change, and its efficiency in channeling DOC (energy) to higher trophic levels will decrease (indicated by the shapes of the corresponding figures in Fig. 15). Generally, the trophic transfer efficiency of the microbial loop is lower compared to the classical food chain (phytoplankton → mesozooplankton → fish) (Suikkanen et al. 2013, Andersson et al. 2015), but this efficiency might further decrease if the DIADOM-proportion of the labile DOM pool decreases in the future.

5.2) Seston stoichiometry – Driven by community composition or physiology?

The Chl *a*:C ratio was determined by the total diatom-biomass, irrespective of the dominant diatom species (chapter 1), which could be related to a more rapid Chl *a* synthesis (Ross & Geider 2009), and generally higher contents of this pigment compared to dinoflagellates (Finkel et al. 2010). The variability around the average Chl *a*:C (n = 125, chapter 3) ratio was relatively low, most likely due to the rather stable environmental conditions (for example low temperature). Thus, the presented Chl *a*:C ratio is an appropriate conversion estimate between Chl *a* and seston C in the Baltic Sea surface water during spring, supported by Simis et al. (2017) that presented only a slightly higher estimate (15 %). Therefore, POC can be estimated with relatively high precision (~60 %) based on Chl *a* concentrations in specific seasons (e.g., remote sensing and ferry box measurements), which could be interesting for modeling studies. Thus, the data obtained by existing phytoplankton monitoring programs are valuable for biogeochemistry and food web models with reference to the Baltic Sea ecosystem, especially when including different functional plankton groups (Lehtinen et al. 2016, Fransner et al. 2018). The Chl *a*:C ratio during the Baltic summer is clearly lower (~50 %) compared to the presented spring data (Simis et al. 2017). Including samples from different depths (light conditions) or seasons (temperature and community) would increase the variability in the Chl *a*:C ratio due to variations in pigment concentrations (Chan 1980, Neale et al. 1989). During the cruises, *T. baltica* was the dominant diatom at stations with the highest levels of BSi, and thus, affected the seston C:Si and N:Si ratios, which exponentially decreased with increasing biomass of this large-celled (≤110 µm), strongly silicifying diatom (Olli et al. 2008). Based on studies like the presented one, valuable data can be obtained to model the dynamics in less frequently monitored / sampled seasons, such as the Baltic spring.

Inorganic C, N, P, and silicate are essential elements for phytoplankton amongst other organisms. First and foremost, these macroelements (amongst others) form the basis of organic molecules, which play an important role in cell structure and activity (i.e. cell wall, DNA, proteins) as well as energy-storage (for instance, C-rich fatty acids). Thus, these elements contribute a significant part to the biomass. Additionally, P (in the form of HPO_4^-) is involved in the ATP (Adenosine triphosphate) metabolism, and thus, the production of energy within all living cells (Sterner & Elser 2002). Therefore, it is crucial which forms of these macroelements are available. For instance, N_2 cannot be used by most phototrophic plankton, but dissolved nitrate is readily bioavailable (Collos & Berges 2011).

The Redfield-related seston ratios (C:N:P, Redfield 1958) were affected by the plankton community composition (higher in mixed communities with low diatom-proportions) to rather low and different extents (N:P 28 %, C:P, 20 %, C:N 8 %). These findings (field data) indicate that the growth / bloom phases had a more substantial effect on the stoichiometry. For this summary, the seston stoichiometry was analyzed for the experiment in 2012, which featured clearly different dominance patterns, unlike the more diverse communities in 2013, and during the cruises. *C. wighamii* (at absolute dominance) seems to have higher C:N (more explicit)

and C:P ratios compared to the DinoComplex and more mixed diatom assemblages. The diatom treatment featured a higher Chl *a* concentration than the DinoComplex, which agrees with the trend of the Chl *a*:C ratio in field samples (see above). Especially the C:P ratios at the phytoplankton peak were quite similar in the diatom treatment and DinoComplex (molar ratios: 375 and 450, respectively). Both ratios developed similarly in DinoComplex and the diverse diatom community (low *Chaetoceros* spp.) in the control, and the latter featured lower values around the peak only. The presented results suggest that the variations in seston C were responsible for the different ratios. As mentioned, the genus *Chaetoceros* is known to produce and excrete more labile polysaccharides than other diatoms and dinoflagellates (Myklestad 1995, Thornton 2014), which could explain the higher C content relative to P. On the other hand, the cellulose cell wall of thecate dinoflagellates contains a high proportion of C, whereas diatoms live in a glass house out of silicate, which could explain the lower Chl *a* and similar seston C in the DinoComplex treatment.

The development of seston C:N, C:P, and Chl *a* followed a Gaussian-like temporal trend along the bloom, irrespective of the community composition, which largely agrees with the shown findings from the field combining the data of four cruises. Thus, the development of seston ratios during both experimental and natural blooms was probably due to physiological differences (growth / bloom phases) rather than only the community composition. This is supported by the fact that no significant correlations ($p \geq 0.26$) were found between the DDP (modified after Wasmund et al. 2017) and seston C:N:P ratios, when pooling T0 and peak samples (different biomass) of all treatments (different dominance patterns) and both experiments (n = 14). To rule out a biomass-effect and study group- and species-specific effects, for example, chemostat experiments with similar biomasses and ideally monoalgal diatom- and dinoflagellate-treatments could be done.

Despite the very similar communities, the C:N:P ratios were significantly higher ($p \leq 0.015$) at the peak compared to the growth phase (field data). On the other hand, the C:N:P ratios at the growth and post-bloom phases were quite similar in clearly different communities (Fig. 3, 8, and 15), highlighting the importance of plankton physiology, driven by the same factors affecting the biomass. Martiny et al. (2013) found the lowest C:N:P ratio (78:13:1) in cold, nutrient-rich, high-latitude regions, which agrees with the presented findings. In a seasonal study, elevated C:P and N:P ratios were linked to increasing temperature, decreasing inorganic nutrients (both affecting physiology and biomass), and growing proportions of smaller phytoplankton (Martiny et al. 2016). Thus, increasing temperature and decreasing nutrients could have contributed to increasing C:N:P ratios from growth to peak phase in the presented field data. Furthermore, the cell content of P-rich ribosomes is higher at high growth rates, irrespective of which organisms are growing (Goldman et al. 1979, Toseland et al. 2013), which explains similar seston ratios in different communities / bloom phases. However, zooplankton and heterotrophic bacteria feature higher cell-specific P-contents compared to phototrophs (Andersen & Hessen 1991, Vadstein 2000, Sterner & Elser 2002), having the same effect on C:N:P as actively growing cells. Furthermore, dinoflagellates feature high uptake affinities for P and continue its assimilation even after the growth phase (Kremp et al. 2008). These factors combined contributed to the development of the C:N:P ratios along with the bloom. Small phytoplankton cells (<10 µm) became more important in the decline and post-bloom phases (details in Spilling et al. 2019). The highest average abundance of heterotrophic bacteria was found at the post-bloom phase (data not shown), but due to their size and the used filters, their exact contribution to the presented stoichiometry is unclear (Sieburth et al. 1978). Heterotrophic ciliates (retained on filters) increased gradually from the growth to the post-bloom phase. Dinoflagellates contributed the same high biomass at all bloom phases (~40 %), but their proportion relative to diatoms increased along with the bloom.

5.3) Linking community changes to biogeochemical cycles with implications for climate change

Temperature and concentrations of dissolved inorganic nutrients were the main drivers of the plankton composition in this dataset. Thus, global warming can be expected to affect the community structure further and thereby, modify for instance, food web dynamics (Camarena-Gomez et al. 2019, bioRxiv), and biogeochemical cycles. Increased temperatures leading to increased stratification and decreased nutrient availability in surface waters were predicted for the Baltic Sea region (e.g., Meier et al. 2012, Thomas et al. 2017). The proposed conceptual model (Fig. 15) includes several aspects that are transferable to the effects of global warming. As microzooplankton becomes more relevant in the warm season (Mironova et al. 2012), grazing pressure on phytoplankton and bacteria is likely to increase in the future. In Fig. 15, this is indicated by the increasing proportions of heterotrophs and the decrease in bacterial activity during the secondary production phase. Furthermore, warming is known to favor smaller plankton groups such as picophytoplankton (Morán et al. 2010), affecting the community composition. As heterotrophic bacteria fall within the picoplankton range (0.2-2 µm, Sieburth et al. 1978), picophytoplankton could replace bacteria as food particles, with implications for the microbial loop. If the phototrophic proportion (mainly diatoms) decreases due to global warming (Andersson et al. 2015), also the labile DOM concentration will decrease (Fig. 15), potentially reducing the energy efficiency of the microbial loop. The mesocosm experiments have shown that bacterial activity is higher in diatom-dominated communities. The complexity of the interaction effects that marine ecosystems are facing makes predictive modeling a complicated task. Increasing temperatures are suggested to increase the DOM-excretion by phytoplankton (Thornton 2014), and thus, the DOC concentration fueling bacterial activity (Fig. 15), but species-specific effects should be considered. Especially, dinoflagellates and ciliates could benefit from changing environmental conditions that simultaneously disfavor diatom growth. A recent report that *M. rubrum* will become more abundant in the northern Baltic Sea (Kuosa et al. 2017) and the decreasing DDP in some sub-basins (e.g., Wasmund & Uhlig 2003) support this hypothesis. Due to the different ecologies of, for example, DinoComplex species, their effects on biogeochemical cycles can vary substantially. According to Hjerne et al. (2019), elevated temperatures cause increasing abundances of dinoflagellates and *M. rubrum* over diatoms, increasing the energy transfer from primary to secondary producers in the water column. This would reduce the amount of newly produced OM, available for the benthos, and thus, a decrease in respiration at the seafloor (Hjerne et al. 2019). The PO_4^{3-} dynamics especially in the GOF and the resulting low N:P supply ratios after the spring bloom, are transferable to other parts of the world ocean. For instance, warming causes decreasing N:P supply ratios, which are known to affect biogeochemical cycles and plankton communities in the Peruvian upwelling (Hauss et al. 2012), the tropical Pacific, and Atlantic Ocean (Franz et al. 2012). These conditions are known to favor toxic dinoflagellates (Shi et al. 2005) and diazotrophic cyanobacteria (Franz et al. 2012). Both of these groups are relevant members of the summer community in the Baltic Sea (Kremp et al. 2009, Elmgren et al. 2015). The ongoing eutrophication (Meier et al. 2012b, HELCOM 2018) may cause drastic changes to biogeochemical cycles (e.g., Falkowski et al. 2000), which could also reflect in the C:N:P ratio of the plankton biomass. Variations in the elemental composition of phytoplankton communities may affect, for example, zooplankton growth, food web dynamics, and the remineralization of nutrients, with implications for the global C-cycle, playing a pivotal role in climate change (Sterner & Elser 2002). Heterotrophic bacteria might benefit from the predicted increase in allochthonous OM levels (Andersson et al. 2015). However, the quality could be lower compared to DIADOM. Assuming heterotrophic processes become more critical at the expense of primary production, this could potentially cause a decrease in the O_2 concentration of the atmosphere.

As species-level identification is crucial to understand the interactions between different organisms and the environment entirely, a pilot study was conducted to separate the dinoflagellates included in the DinoComplex. The three species are ecologically different, in

terms of nutrient uptake and encystment strategies (Sundström et al. 2009, Warns et al. 2012). Thus, depending on the dominant species, biogeochemical cycles are affected differently. Preliminary results (Fig. 7) of the pilot study suggest that the three involved species differ in their spatial dominance patterns. *Gymnodinium corollarium* was most abundant in the BP, where this species was initially found (Sundström et al. 2009). In contrast, *Biecheleria baltica* formed higher biomass in the GOF and BS. This species can be abundant under the ice before the spring bloom (Sundström et al. 2010), explaining its positive correlation with lower temperatures. The GOF is a highly eutrophicated sub-basin, supporting high algal biomass in general (Pitkänen et al. 2001). A study on a 100-year-old sediment core from the GOF (Kremp et al. 2018), revealed that drastically increasing abundances of *B. baltica* cysts coincided with increasing eutrophication since the 1930´s, supporting the shown findings from the pelagic. *Apocalathium malmogiense*, which was thought to be a bloomer as well, contributed only a minor fraction of the dinoflagellate biomass, highlighting the importance of species-level identification. These findings agree with the cyst records from sediments as well (Kremp et al. 2018). The pilot study has shown that different methods can be used to unravel the species-specific contributions of the three, by conventional light microscopy inseparable, dinoflagellates. The identification of these species would be very valuable for studies on long-term phytoplankton trends to improve the understanding of ecosystem-wide effects of changing communities in the Baltic Sea. Thus, it is highly recommended to include the microscopy method (Fritz & Triemer 1985) in phytoplankton monitoring programs. This technique could be applied to the same samples as currently used for phytoplankton identification and quantification (minding the pH of the preservative), is rather inexpensive, and relatively easy to learn. The qPCR method using species-specific primers and Taqman®-probes (Brink et al. 2019, bioRxiv) is more expensive, but also more sensitive in detecting the desired species (Fig. 7).

6.) Conclusions, outlook, and take-home messages

Conclusions

Diatoms play a key role in pelagic-benthic-coupling, are a high-quality source of labile DOM for heterotrophic bacteria, and important marine primary producers. Thus, a further decrease in the diatom-dinoflagellate-proportion (DDP) would negatively affect the amount of DIADOM for heterotrophic organisms, the energy efficiency of the microbial loop (potentially lower labile DOC), and the aquatic oxygen production. Furthermore, diatoms grow best at low temperatures, high nutrient concentrations (and N:P supply ratio) in the surface water, and mixed conditions. Global warming is predicted to change all these factors, and thus, the DDP could decrease further in the future. The interactions of different organisms with the environment and each other are highly dependent on the growth phase, and thus, cell physiology, which is supported by the fact that high biomass of the diatom *Thalassiosira baltica* at the bloom peak was associated with both low primary production (presented study) and low bacterial production (data not shown). This was probably related to the fact that *T. baltica* was already less active, but the cells were not decaying, which would boost the bacterial activity. Species-specific effects of different diatoms on bacterial responses were observed, indicating that the species composition within different groups should be considered to study the sensitivity of ecosystem functioning to community changes, besides different proportions of varying plankton groups. In general, the observed species succession along the natural bloom, agrees with previous studies. What is interesting is that four species / groups jointly dominated the biomass considering 119 stations across the Baltic Sea: *Mesodinium rubrum*, *Peridiniella catenata*, DinoComplex, and heterotrophic ciliates, implying a low proportion of diatoms considering data from four subsequent spring blooms. These findings agree with their decline in some Baltic Sea sub-basins observed in the last decades. Both dinoflagellates and ciliates are favored by elevated temperatures and have very different

ecological strategies, affecting nutrient dynamics as well as community structure and processes. Thus, adjusting monitoring programs to follow changing food web dynamics (for example, increased grazing pressure on phyto- and bacterioplankton) in a changing ocean are crucial. The fact that only the late phase of the spring bloom was covered by monitoring programs until recently indicates the significance of extensive datasets from this most productive season of the year.

Comparing the presented seston stoichiometry data from field studies to the literature is quite tricky, since studies on the entire spring bloom in the Baltic Sea seem rare. However, the comparison to mesocosm studies (literature and chapter 2), revealed a fundamental observation: absolute dominance patterns of either diatoms or dinoflagellates lead to more apparent trends compared to mixed communities with higher diversity. However, in both cases (field study and experiments), the collective physiology of the communities superseded the effect of different dominance-patterns on the seston C:N:P ratios. A cells physiology is affected by environmental factors such as temperature and the concentration of inorganic nutrients. The observed dynamics of the seston C:N:P ratios along the bloom followed similar temporal trends as described by previous studies (Gaussian-like curve). To reliably study the effect of different organisms on biogeochemical cycling of C and nutrients, a different study design should be considered. Species-specific effects can only be reliably investigated in monoalgal treatments under more controlled conditions (e.g., similar biomass), such as an experiment on *Chaetoceros wighamii* by Spilling et al. (2015), to avoid interaction effects, dominating in natural communities, and rule out biomass-effects. The seston ratios that are clearly affected by the community composition, namely Chl *a*:C, C:Si, and N:Si could be predicted with relatively high certainty. The upper limit of the Chl *a*:C ratio during the spring bloom (0.04) and the clear correlation between C:Si / N:Si and diatom-biomass could be interesting for modeling studies. However, data from different seasons include a high variability. Thus, season-specific findings should be considered to predict, for example, the annual primary production considering the net-outcome of phototrophic / heterotrophic processes. Higher respiration at the expense of primary production could contribute to the ongoing environmental changes by affecting the amount of oxygen transferred from the sea to the atmosphere.

Outlook

Our data support previous studies on phytoplankton-bacteria-interactions and suggest that the predominant phytoplankton group (example *Chaetoceros*) defines the predominant type of DOM available for heterotrophic growth, representing different niches for different classes of bacteria. Experiments with similar biomass and only one phytoplankton species per treatment could be conducted to study the effects of absolute dominance patterns (diatoms vs. dinoflagellates) and species-specific effects on natural bacterial assemblages of the Baltic Sea. This would reduce the microscopy work and leave room for the chemical analyses of the labile DOM pool, amongst others. Additionally to community effects on seston C.N:P ratios, it would be interesting to disentangle the interaction effects of abiotic factors (such as temperature and inorganic nutrient supply) on biogeochemical cycles, using the presented dataset. As heterotrophic bacteria and picophytoplankton are suggested to benefit from climate change, their contribution to seston stoichiometry might increase as well. Even though the contributions of detritus and picoplankton to the seston are considered negligible during the spring bloom (Lipsewers & Spilling 2018; Wasmund 2020, personal communication), it would be very interesting to disentangle the exact effects of all relevant seston compartments in future studies. By filtering different size fractions of natural seawater, it could be studied how for instance phytoplankton stoichiometry is reflected in heterotrophic biomass and how phototrophic proportions will develop in the future, with implications for nutrient- and C-cycles as well as food web dynamics. Dinoflagellates are a special case: during the spring bloom the dinoflagellate community comprises species that are a source of DOM for bacteria, but

also species that feed on bacteria or repel them by producing allelopathic compounds (not part of presented study). In this case, it is even more important to identify the species correctly (for example, epifluorescence microscopy of Calcofluor white MR2 stained cells) before establishing a cell line of natural isolates and consider mixed and / or monoalgal treatments, depending on the research question. This is also valid to study species-specific effects on other variables such as seston nutrient stoichiometry. Furthermore, there are data and samples from dilution experiments (field studies) to estimate plankton growth rates and grazing pressure. As heterotrophic ciliates are essential grazers of phytoplankton and bacteria in the warmer season, increasing grazing rates can be expected in the future, indicating the scientific value of quantifying these interactions. However, mainly because of trophic cascades present in natural communities and the fragility of microzooplankton, this is not trivial.

Take-home messages

- The phytoplankton community composition is important in many aspects.
- Considering larger groups (for example dinoflagellates and diatoms) only, will mask species-specific effects.
- The species included in the DinoComplex feature species-specific distribution patterns and occupy different niches during the spring bloom. How do these different dominance patterns affect, for example, biogeochemical cycles?
- The mixotrophic ciliate *M. rubrum* is a significant biomass contributor, but its role in the pelagic food web is still uncertain.
- Diatoms are negatively affected by warming, which could have significant ecological implications. Generally, they provide higher amounts and higher quality of DOC for bacteria, have a higher Chl *a*:C ratio, and sink faster than dinoflagellates.
- Many factors affect seston stoichiometry: cell physiology, community composition, and the proportion of detritus to the total seston, amongst others.
- Even though the Baltic Sea is a unique ecosystem, the consequences of climate change and eutrophication are transferable to, for instance, the large upwelling areas of the world ocean.

My personal take-home message

From my studies and the literature review, I learned that sustainability on private, professional, and more geologically relevant levels will be important projects in the future. Regarding the conservation of the Baltic Sea ecosystem and other marine habitats: I hope that mitigation strategies (especially against eutrophication) will become more effective in protecting marine ecosystems in the long-term. Regarding my own future: Studying the base of the food web and how it is affected by environmental changes, and particularly the performance of a Ph.D. study, made me reflect on many aspects. Soon, I would like to optimize and publish a new qPCR method for the identification of the DinoComplex species and finalize unraveling their contributions and dynamics during the spring bloom. Furthermore, I would like to apply what I learned from plankton and the interactions of abiotic and biotic factors within the aquatic food web to the field of aquaponics.

7.) Acknowledgments

The presented study utilized the Finnish Environment Institute Marine Research Centre (SYKE MRC) infrastructure as a part of the national FINMARI RI consortium. The research for this thesis was conducted at SYKE MRC and Tvärminne Zoological Station (University of Helsinki). The fieldwork was done aboard R/V Aranda, and 4 research cruises were conducted, 3 of which I had the pleasure to participate. The FINMARI culture collection (former SYKE MRC culture collection) kindly provided the dinoflagellate strains used for qPCR and epifluorescence microscopy. The Academy of Finland and the Onni Talas Foundation provided the funding for the largest part of this project. The SYKE MRC covered the costs for printing this thesis. Thank you very much for this great support!

I am truly grateful for the comments of my pre-examiners (Inga Lips and Norbert Wasmund). Thank you very much for your thorough reading, very constructive advice, and improving the quality of my thesis. The support of my thesis advisory committee (Anke Kremp and Magnus Lindström) was essential. Anke: You gave me ideas for new studies, shared your network of scientists with me, and made me a better researcher. Ich danke Dir vielmals! Magnus: I benefitted from your vast experience as a scientist and enjoyed our pleasant and helpful meetings every time. Kiitos paljon / Tack så mycket! A big thank you to Angela Wulff, who accepted to act as my opponent. A gigantic thank you goes to my supervisors (Kristian Spilling and Harri Kuosa). Kristian: You gave me the opportunity to be part of this interesting project. You gave me freedom and space to make my own mistakes and learn how to become a scientist. Even though we had our differences, in the end, we made it work together. You were always available for discussions and advice. Keeping in mind what I would have missed out on without working in this project and coming to Finland, I cannot thank you enough. Tussen takk! Harri: It was a pleasure to work with you and to learn from you. Your support was essential, and I would not be at this point without your supervision. I cannot express in words how vital your contribution was for my thesis and how grateful I am. Kiitos paljon!

Special thanks go to all my co-authors listed on the different publications. Without you, this achievement would not have been possible. I am more than grateful for your contributions. Thanks to the staff of the “nutrient team” of SYKE MRC (most importantly Pia, Ilkka, Kirsi) and Tvärminne Zoological Station for their support during the cruises and experiments. I am especially thankful for the collaboration with the R/V Aranda crew, which provided crucial technical support and excellent catering during the cruises. The contributions of some people need to be emphasized. Johanna Oja: Thank you so much for the microscopy work (cruises in 2015/16). Without you I would still sit in front of the microscope ;) It was a real pleasure to work with you. Alicia Martinez-Varela: Muchas gracias for your help, the fun, and the conversations during the experiment in Chile (2014), and the cruise in 2016. It was a pleasure to meet you. And don´t forget “Viva la …!” Riina Klais: I am particularly grateful for your support regarding the multivariate statistics (chapter 1) and the days I spent in Tartu to learn from you. Your contribution was vital, and you R a genius. Mira, Amelia, and Arto: You have done an excellent job with the flow cytometer. Thank you for your hard and accurate work. Henna Savela: Our collaboration was rather short, but I learned so much from you about qPCR and molecular biology in general. Thank you very much. The outcome of the Chile experiment was not part of my thesis, but: Thank you sooo much, Vivian Montecino! You opened your house to us, and it was more than a pleasure to meet you and learn from you. You have a special place in my heart. The staff and researchers of the field station in Las Cruces: Thank you very much for contributing to this great experience. Cristina Sobrino: I am more than thankful to have met you and for your positive attitude.

During the years, I have seen some leaders of the Marine Ecosystems Modeling group of the SYKE MRC. Special thanks go to Risto Lignell, Timo Tamminen, and Harri Kuosa for open ears, open minds, and open doors. Thank you very much also to Ritva Eloranta and Anne

Manninen for their essential support regarding the administration during my contract with SYKE.

Additionally, I am privileged to have met and interacted with many fantastic people during the past seven years: Danielle, Samu, Mari, Kata, Riikka, Jonna, and Anne-Mari to name some. You have truly enriched my Ph.D. life, and it was a great pleasure to share conversations, ideas, and problems. Thank you very much. Special thanks go to Samu and Mari, who provided the Finnish version of the thesis abstract. Tere: I come back to you later. Special thanks also go to Sakari Kuikka (Professor in Aquatic Sciences, U Helsinki), who stood up for me in the Faculty Council Meetings and was a great support during the submission and examination process of this thesis. Kiitoksia paljon!

At least one million thanks go to my family:
Maritheres, Reimund, Franz-Josef, Yvonne, Klausi, Hildegard & Heinrich (you are an angel and I will never forget you), and the latest member, Jacqueline. Without (all of you) I´m nothing! Not only that, you always listened to me and supported me no matter what, especially Yvonne & Jacqueline also contributed actively to the improvement of my publications and my thesis. Jacqueline: It was a rollercoaster ride ever since we met, and I´m delighted that we ride on to the next looping. I love all of you, and I owe you my life.

…and my "Finnish" family:
Tere & Carlos with Luca & Orejas, Omar, Timo, Dimitra, Atte, Duki, my Sauna crew, my fishing crew, my football crew. Without you, I would not have managed to complete this project. I am deeply thankful to have met you, and I love all of you. You are my heart and my soul. Kiitos miljoona! Tere: As you mentioned in the acknowledgments of your thesis, it was not all smooth sailing. After all, I am truly grateful for writing chapter 2, for all up´s and down´s (they made me grow personally and professionally), for being my colleague, my friend, and my Ph.D. sister. Muchas gracias! Omar: You are a special case. I admire your passion and your creativity. You are a genius. The cover you designed is just beautiful. I cannot thank you enough. Muchas gracias Qsho! Atte: My brother in spirit. You are the center of my social life in Finland. You showed me one of the most beautiful regions of the world, Finnish Lapland (from Norvajärvi to Inarijärvi). You made me feel like a member of your family. I am speechless. Kiitos Kiitos Kiitos! You all motivated me to complete my Ph.D.

I would also like to thank radioparadise.com (Rebecca and Bill) for providing the soundtrack of my life for 10 years already. Music is the best therapy you can get. Viva la musica!

As I would like to cite dozens of quotes in addition to Coldplay, I use the opportunity to add one here:
„Wer noch nie einen Fehler gemacht hat, hat sich noch nie an etwas Neuem versucht."
Albert Einstein (1879 - 1955)

Thank you, mother earth!

I sincerely hope that I have not forgotten anybody. If I did: THANK YOU VERY MUCH!

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9.) Supplementary materials

The supplementary materials for chapter 1 and 2 are available digitally (chapter 1: https://doi.org/10.3354/meps13361, chapter 2: https://www.int-res.com/articles/suppl/a081p149_supp.pdf). Additional information regarding this summary can be found in the following.

Dinoflagellate identification by qPCR

The target sequences for amplification belong to the internal transcribed spacer (ITS) region 1 in the case of *B. baltica* and ITS 2 in the case of *G. corollarium* and *A. malmogiense*. Both ITS regions belong to the ribosomal gene complex and feature a high genetic deviation, which makes them a suitable biomarker to distinguish between different species. The DNA-extractions were initiated by pretreating the samples (50 ml filtered) with a harmless resin (Chelex®) and beating with glass beads. Subsequently, DNA contents and purity were determined (NanoDrop®). Species-specific primer pairs and custom-made Taqman® probes were used to increase the specificity of the method. Each qPCR-plate included at least three non-template controls (NTC: nuclease-free water instead of template DNA), which should not result in an increase of fluorescent PCR-product, to ensure the reliability of the reaction and the purity of reagents. An individual PCR-plate was prepared for each species. The species-specific mastermix (primers, probes, *etc*.) was prepared and added to a 96-well plate, and templates as well as non-templates were added subsequently. Plasmid DNA of cultured strains was used for species-specific standard curves.

Obstacles

The primary concern about this method is the undesired amplification of non-target DNA in the mentioned positive and negative controls. Results show that all three primer-pairs targeted the DNA of more than one species, pointing out the urgent need for the development of 100 % species-specific primer sets. Several reasons can be given for the unspecific binding of the used primer sets. The contamination of solutions has been checked by testing fresh dilutions and aliquots. Anyway, something could have been wrong with the reagents already before delivery. A contamination of reagents can most likely be ruled out, since the NTC´s were not problematic. Furthermore, a contamination of the cultures, used as controls, was suggested as a possibility, but epifluorescence microscopy revealed that the same strains were monoalgal. qPCR products of samples and controls were checked by gel-electrophoresis for unspecific bands at random. Generally, qPCR-efficiencies of 84-104% were achieved. The correlations of the results obtained by both methods (Fig. 6), revealed that the unspecific amplification of cultured strains did not affect the species identification in the mixed environmental samples.

Trouble-shooting

Gel-electrophoresis and sequencing verified unspecific amplifications. None of the tests (for example using defined DNA concentrations, different dilutions of culture DNA, and a different mastermix to prepare melt curves), the preparation of fresh dilutions of primers, probes, solutions, and the extraction of fresh DNA from cultures for new controls resulted in an improvement. Thus, this study was discontinued for now.

Outlook

The development of new primer pairs for the three species is urgently needed. Furthermore, the method could be modified by using SYBR Green as a fluorescent dye for the quantification of PCR-products, which would be easier to handle (e.g., not light-sensitive) and less costly. Also, analyzing all species at once (multiplexing) could be considered since it would reduce procedure steps, and thus, potential sources of error. Anyway, cross-contaminations of DNA from different species would be a higher risk. The development of a new method would require the testing and optimization of the new reagents and the type of qPCR-machine used. Additionally, it is possible to linearize the plasmids used for the standard curves, which is suggested to yield higher PCR-efficiencies. However, the qPCR findings correlated well with those of the microscopy approach. Minding the mentioned modifications, it is planned to continue this study using the existing data and further samples. Recently, new primers for *Biecheleria baltica* were developed (Savela et al. 2019, unpublished), which will be very helpful to start the improvement of the protocol.

ISBN 978-951-51-6285-4 (PRINT)
ISBN 978-951-51-6286-1 (ONLINE)
ISSN 2342-5423 (PRINT)
ISSN 2342-5431 (ONLINE)
http://ethesis.helsinki.fi

HELSINKI 2020